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Geneticky podmíněné faktory progrese vybraných chronických nefropatií

Genetically determined progression factors of selected chronic nephropathies

Disertační práce

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ABSTRAKT

Polycystická choroba ledvin je závažné geneticky podmíněné onemocnění vyskytující se u dospělých i dětských pacientů. Základní charakteristikou tohoto onemocnění je vznik a postupné zvětšování renálních cyst, které nahrazují funkční tkáň ledvin. U řady pacientů tak dochází k renálnímu selhání. Renální cysty se ovšem mohou vyskytovat i u řady dalších onemocnění, včetně multisystémových syndromů. To u některých pacientů komplikuje diferenciální diagnostiku onemocnění. V naší studii jsme se nejprve soustředili na diagnostiku a charakterizaci genotypově-fenotypových souvislostí u pacientů s polycystickou chorobou vznikající v dětském věku, později jsme naši studii rozšířili i na dospělé pacienty a pacienty s nejasnou klinickou diagnózou. Zároveň jsme zvětšili portfolio analyzovaných onemocnění, a to na řadu nemocí, u nichž se může vyskytnout fenotyp polycystických ledvin, i na onemocnění necystická.

Během našeho projektu jsme metodou masivního paralelního sekvenování analyzovali 149 pacientů – 128 s cystickými a 21 s necystickými klinicky diagnostikovanými nefropatiemi. Zároveň byly nálezy ověřeny Sangerovou sekvenací u 176 příbuzných našich probandů. Mutační detekce dosahovala 59% u cystických pacientů a 43% u necystických pacientů. U řady pacientů molekulárně genetická analýza odhalila jinou etiologii onemocnění, než bylo klinicky indikováno.

Klíčová slova:

Polycystická choroba ledvin, sekvenování nové generace, PKHD1, PKD1, PKD2

ABSTRACT

Polycystic kidney disease is a severe genetic disease occurring in both adult and pediatric patients. The basic characteristic of this disease is the development and progressive enlargement of renal cysts gradually replacing functional kidney tissue. This leads to renal failure in many patients. However, renal cysts may also occur in a number of other diseases, including multisystem syndromes. This complicates differential diagnosis in some patients. In our study, we first focused on the diagnosis and characterization of genotypic-phenotypic relationships in patients with polycystic disease arising in childhood, later we extended our study to adult patients and patients with unclear clinical diagnosis. At the same time, we expanded the portfolio of analyzed disorders to a number of diseases in which the phenotype of polycystic kidneys may occur, and noncystic diseases as well.

During our project, massive parallel sequencing was used to analyze 149 patients – 128 with cystic and 21 with noncystic clinically diagnosed nephropathies. At the same time, the findings were verified by Sanger sequencing in 176 relatives of our probands. Mutation detection reached 59% in cystic patients, and 43% in non-cystic patients, respectively. In many patients, molecular genetic analysis revealed a different etiology of the disease than clinically indicated.

Key words:

Polycystic kidney disease, next-generation sequencing, PKHD1, PKD1, PKD2

ABBREVIATIONS

ACKD	Acquired cystic kidney disease
AD	Autosomal dominant inheritance
ADPKD	Autosomal dominant polycystic kidney disease
ADPLD	Autosomal dominant polycystic liver disease
ALG8	ALG8 alpha-1,3-glucosyltransferase
AP-1	Activator protein 1
AR	Autosomal recessive inheritance
ARPKD	Autosomal recessive polycystic kidney disease
Array CGH	Array comparative genomic hybridization
BBS	Bardet-Biedl syndrome
BOR	Branchiootorenal syndrome
CKD	Chronic kidney disease
CNS	Central nervous system
CSV	Comma-separated values file
CTT	Carboxy-terminal tail
DNA	Deoxyribonucleic acid
DNAJB11	DnaJ heat shock protein family (Hsp40) member B11
DZIP1L	DAZ interacting zinc finger protein 1 like
ECM	Extracellular matrix
EMA	European Medicines Agency
ER	Endoplasmic reticulum
ERA-EDTA	European Renal Association - European Dialysis and Transplant Association

ERK	Extracellular signal–regulated kinase
ESRD	End-stage renal disease
FDA	U.S. Food and Drug Administration
GANAB	Glucosidase II alpha subunit
GFR	Glomerular filtration rate
GPCR	G protein-coupled receptor
GPS	G-protein coupled receptor proteolytic site domain
GRCh38	Genome Reference Consortium Human Build 38
GSK-3	Glycogen synthase kinase 3
GTP	Guanosine-5'-triphosphate
HNF1B	HNF1 homeobox B
Id2	Inhibitor of DNA binding 2
IFT	Intraflagellar transport
IP_3R	Type I Inositol 1,4,5-triphosphate receptor
IPT	Ig-like, plexins, transcription factors
JAK	The Janus kinase
JNK	c-Jun N-terminal kinase
KDIGO	Kidney Disease: Improving Global Outcomes
LRP	Prolow-density lipoprotein receptor-related protein
LRR	Leucine-rich repeat
LVH	Left ventricular hypertrophy
MDCK	Madin-Darby canine kidney cells
MDCK/FJHN	Medullary cystic kidney disease 2/familial juvenile hyperuricemic nephropathy
MID	Multiplex identifier

MLPA	Multiplex ligation-dependent probe amplification
MNP	Multi-nucleotide polymorphism
MODY	Maturity-onset diabetes of the young type
mTOR	Mammalian target of rapamycin
NFAT	Nuclear factor of activated T-cells
NGS	Next-generation sequencing
NLS	Nuclear localization site
NPHP	Nephronophthisis
OFD1	Orofaciodigital syndrome 1
ORF	Open reading frame
PACS	Phosphofurin acidic cluster sorting protein
PBS	Phosphate-buffered saline
PC1	Polycystin-1
PC2	Polycystin-2
PCR	Polymerase chain reaction
PI3K	Phosphatidylinositol 4,5-bisphosphate 3-kinase
PIP2	Phosphatidylinositol 3,4-bisphosphate
PIP3	Phosphatidylinositol 3,4,5-trisphosphate
PKB	Protein kinase B
PKC	Protein kinase C
PKD	Polycystic kidney disease
PKD1	Polycystin 1, transient receptor potential channel interacting
PKD2	Polycystin 2, transient receptor potential cation channel
PKHD1	PKHD1 ciliary IPT domain containing fibrocystin/polyductin

PLC	Phospholipase C
PLD	Polycystic liver disease
PRKCSH	Protein kinase C substrate 80K-H
RCAD	Renal cysts and diabetes syndrome
REJ	Receptor for egg jelly domain
Rheb	Ras homolog enriched in brain
RRT	Renal replacement therapy
SBS	Sequencing by synthesis
SEC61B	SEC61 translocon beta subunit
SEC63	SEC63 homolog, protein translocation regulator
SFF	Standard flowgram format
SNP	Single-nucleotide polymorphism
SRTD	Short-rib thoracic dysplasia with or without polydactyly
STAT	Signal transducers and activators of transcription
STAT6	Signal transducer and activator of transcription 6
TCF	T cell factor
TCF2	Transcription factor 2
TM	Transmembrane
TOP	Tetragonal opening for polycystins
TRP	Transient receptor potential
TSC	Tuberous sclerosis complex
US	Ultrasound
VCF	Variant Call Format file
VEO	Very early onset

VSLD	Voltage-sensor-like domain
VUR	Vesicoureteral reflux
XLD	X-linked dominant inheritance

INTRODUCTION

Autosomal dominant polycystic kidney disease

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited disorder that typically presents in adults. It is a multisystem disorder that manifests with gradually growing fluid-filled renal cysts that start to develop in utero and usually originate from distal regions of the nephron and collecting ducts. ADPKD manifestations also comprise extrarenal changes, such as hepatobiliary and cardiovascular abnormalities. Although the ADPKD is a monogenic disorder, it is genetically and allelically heterogeneous with different range of severity and phenotypic manifestation.

It is believed that the clinical manifestations of polycystic kidney disease (PKD) might be noted by Hippocrates (born 460 BC) – as his description of fourth disease of the kidney seems to encompass (with a little imagination - as mentioned by authors of the article Torres and Watson, 1998) polycystic kidney disease. With the rising number of autopsies in 16th and 17th centuries, the cysts located in the kidneys were described by several authors (see Torres and Watson, 1998). Nevertheless, PKD did not caught the attention of physicians until the 18th century. In 1793, Dr. Matthew Baillie noted in his anatomy book "Morbid Anatomy of Some of the Most Important Parts of the Human Body" (Balat, 2016) *"hydatids of the kidney"* that can be so numerous *"the natural structure of a kidney is almost entirely lost, and is changed into a mass of small hydatids. When this is the case, the mass is commonly much larger than the natural size of a healthy kidney"*. The kidney failure caused by bilateral cystic degeneration was recognized by Pierre François Olive Rayer in his "Traité des maladies des reins" published in 1840 (Rayer, 1840). Nevertheless, the term "polycystic kidney" was not used until 1888 by Félix Lejars in his doctoral thesis (Balat, 2016; Torres and Watson, 1998).

Epidemiology

Prevalence of ADPKD based on the two early studies was estimated to be 1/400 to 1/1,000 live births (Dalgaard, 1957; Garcia Iglesias et al., 1983). On the basis of this prevalence, ADPKD would affect about 12.5 million people worldwide (Chebib and Torres, 2016). Nevertheless, recent studies show lower prevalence of ADPKD ranging

from 3.96/10,000 based on the data from European Renal Registry (Willey et al., 2017), 4.07/10,000 in the British study (Daviest et al., 1991), 4.76/10,000 in Italian study based on epidemiological data (Solazzo et al., 2018) to higher prevalence of 9.3/10,000 estimated in study using measurement of frequency of high-confident mutations of ADPKD genes (*PKD1* and *PKD2*) in databases gnomAD and BRAVO (Lanktree et al., 2018), or autopsy-based studies showing prevalence of ≥ 1:500 (Chan, 1993; Dalgaard, 1957; Garcia Iglesias et al., 1983). The higher estimated prevalence detected in populations analyzed by massive parallel sequencing or with autopsy-based analyses can indicate that number of affected patients (probably with mild phenotype of ADPKD) remain undiagnosed. Regarding racial differences, non-Hispanic blacks are less likely to have end-stage renal disease (ESRD) attributed to ADPKD compared to non-Hispanic whites. Nonetheless, ESRD from ADPKD appears at younger age in non-Hispanic blacks (54.4 years ±13) that in non-Hispanic whites (55.9 years ±12. 8) (Murphy et al., 2019).

Clinical manifestation

ADPKD is characterized by formation and progressive growth of renal cysts producing gradual kidney enlargement and replacement of normal renal parenchyma causing chronic kidney disease (CKD, loss of kidney function) and ultimately resulting in end-stage renal disease (ESRD, renal failure). ESRD occurs in up to 75% of ADPKD patients by 70 years of age causing necessity for renal replacement therapy (RRT) (Neumann et al., 2013). Hence ADPKD patients form about 10% of patients in dialysis and transplantation programs (Spithoven et al., 2014). ADPKD is a systematic disease and patients often develop extrarenal manifestations, such as polycystic liver disease (PLD), cysts in the pancreas, seminal vesicles and the arachnoid membrane, and cardiovascular abnormalities including hypertension, left ventricular hypertrophy, aneurysms and cardiac valvular abnormalities.

Renal manifestations include formation of cysts originating from all parts of nephron but predominantly from distal regions (Grantham et al., 1987) (Figure 1). They arise from the minority of nephrons and gradually detach from the tubule segment (Grantham et al., 1987). Gradual growth of cysts due to abnormal cell proliferation, fluid secretion and production of extracellular matrix causes progressive loss of functional renal parenchyma associated with decrease of glomerular filtration rate (GFR, with associated proteinuria used as a prognostic marker of ADPKD) and end-stage renal disease (ESRD). However,

measurement of total kidney volume is used instead of glomerular filtration rate for monitoring of disease progression, as GFR can remain within normal range for decades thanks to the elevated filtration of remaining nephrons, but total kidney volume reflects progressive cyst growth in patient (Grantham et al., 2006).

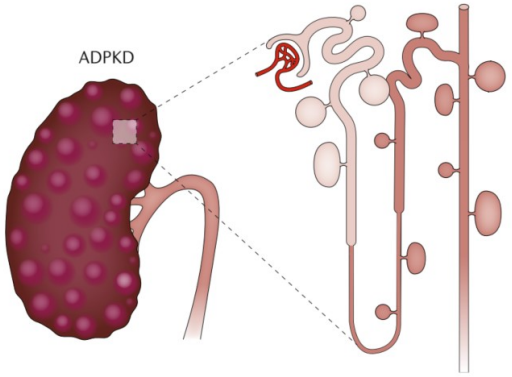


Figure 1: Cystic outgrowths originating in all parts of nephron (mainly distal regions) in ADPKD kidney. From Bergmann et al., 2018.

Common manifestation of ADPKD is hypertension. It is reported in about 50 to 75% of patients and can occur in an early age of patient with ADPKD (Gabow et al., 1990a). Hypertension is associated with progression to kidney failure as well as cardiovascular comorbidities (Schrier et al., 2014). Early detection and treatment of high blood pressure is therefore of great importance for preservation of kidney function and prevention of mortality from cardiovascular abnormalities.

Majority of patients suffer with pain. The causes of pain include cyst enlargement, cyst rupture, infection or nephrolithiasis (Bajwa et al., 2004). The most common types of pain are back and abdominal pain and can be sometimes the actual symptom that leads to the diagnosis of ADPKD (Bajwa et al., 2004).

Another important symptom occurring in about 20% of ADPKD patients is nephrolithiasis (renal stone disease) (Torres et al., 1993). The stones are usually composed by uric acid and/or calcium oxalate (Torres et al., 1993).

Other manifestations of ADPKD include urinary tract infection as well as cyst infections (only in about 9% of patients (Sallée et al., 2009)), hematuria (associated with cyst hemorrhage, infection, or passage of kidney stones) or renal cell carcinoma (Bergmann et al., 2018).

The most common extrarenal comorbidity accompanying ADPKD is polycystic liver disease with prevalence 58% in patients aged 15 to 24 years, 85% in patients aged 25 to 35 years and 94% in age group of 35 to 46 years (Bae et al., 2006). Overall, the prevalence of PLD in ADPKD patients is about 83% without significant differences between men and women (79% and 85%, respectively), lower in young patients and increasing with age. On the other hand, severity of PLD differs between sexes. It has been long known, women are more likely to develop more severe form of PLD (more than 15 cysts) with more massive liver cysts (4.2 ± 0.4 cm) compared to men (2.7 ± 0.3 cm, $p < 0.004$) (Gabow et al., 1990b). Moreover, cyst volume growth is significantly higher in women after multiple pregnancies and/or use of hormonal replacement therapy (Chapman, 2003; Gabow et al., 1990b). PLD is typically clinically asymptomatic and benign, but with lengthened lifespan of ADPKD patients (thanks to RRT and transplantation) the symptoms related to enlarged mass of cystic liver can cause mechanical discomfort, such as lower back pain, dyspnea (shortness of breath), early satiety and dyspepsia (indigestion with symptoms that include bloating, nausea or burping). Complications of PLD including compression of vena cava, cyst hemorrhage, cysts infection and rupture are not as common (Gevers and Drenth, 2013; Hoevenaren et al., 2008; Torres and Harris, 2009).

Cardiovascular abnormalities are common in ADPKD patients and are the common cause of death in ADPKD patients and healthy population. A frequent early symptom of ADPKD is presence of hypertension occurring in patients even before the decline of GFR (Sans-Atxer et al., 2013). The median age of diagnosis of hypertension is in ADPKD patients 32 and 34 years of age for males and females, respectively (Schrier et al., 2003). Furthermore, studies reported hypertension can occurs even in children with ADPKD (Cadnapaphornchai et al., 2008; Ivy et al., 1995). The presence of hypertension in patient is also dependent on family history of ADPKD. It was described that frequency of hypertension was higher in offspring of hypertensive parent that the one of a normotensive parent (Schrier et al., 2003). Hypertension is associated with progression to kidney failure as well as cardiovascular comorbidities (Schrier et al., 2014). Early detection and treatment of high blood pressure is therefore of great importance for preservation of kidney function and prevention of mortality from cardiovascular abnormalities.

Another extrarenal defect that can arise in ADPKD patients is intracranial aneurysm. Occurrence of intracranial aneurysms is elevated in ADPKD patients to 9-12% compared

to 2-3% in general population without any comorbidity (Vlak et al., 2011; Xu et al., 2011). Nonetheless, risk of intracranial aneurysms rupture is not higher in ADPKD patients in comparison to general population (Brown et al., 2011) unless there is a positive history of ADPKD in the family, in which case the risk of rupture is five times higher (Pirson et al., 2002; Torres, 2000).

An early complication recognized in ADPKD patients is also left ventricular hypertrophy (LVH). The clinical outcome of LVH in patient includes increased risk of cardiac arrhythmias, systolic and diastolic dysfunction and sudden cardiac death (Glassock et al., 2009). LVH is an adaptive response to hypertension (Alam and Perrone, 2013) and correlation between hypertension and increased left ventricular mass index (LVMI) has been shown in both adult (Chapman et al., 1997; Schrier et al., 2002) and young (Cadnapaphornchai et al., 2008; Ivy et al., 1995) patients with ADPKD. Studies have also reported that even normotensive ADPKD patients have increased LVMI compared with controls (Chapman et al., 1997; Saggar-Malik et al., 1994). Nevertheless, the results of HALT-PKD study – the prospective randomized double-blind placebo-controlled multicenter interventional trials testing two types of drug therapy (combination therapy of lisinopril plus telmisartan vs monotherapy of lisinopril plus placebo) on disease progression showed low percentage of hypertensive ADPKD patients with LVH (Perrone et al., 2011). In this study, 558 hypertensive ADPKD patients (participants of study A) – underwent magnetic resonance imaging assessment of LVM, renal blood flow and total kidney volume before study intervention. The prevalence of LVH in these patients was unexpectedly low ranging from 0.7% to 3.9%. This could be caused by the fact that antihypertensive treatment was common prior to start of the trial and was probably more aggressive (with lower target blood pressure) than in previous studies.

Cardiac valvular abnormalities are detected in about 25 to 30% of patients with ADPKD. The most common is mitral valve prolapse and regurgitation (in about 26% of patient), following by tricuspid valve prolapse (about 15%) (Hossack et al., 1988). The valvular abnormalities can progress to the point the valve replacement is needed (Leier et al., 1984).

Diagnosis of ADPKD

The diagnosis of ADPKD is primarily based on ultrasound findings (US), and is established if:

1. Patient fulfils the age-specific ultrasound (or MRI) criteria and has an affected first-degree relative with ADPKD
   - Patients aged 15 to 39 years: identification of three or more (unilateral or bilateral) renal cysts
   - Patients aged 40 to 59 years: identification of two or more cysts in each kidney
   - Patients older than 60 years: identification of four or more cysts in each kidney

   Conversely, fewer than two renal cysts in at-risk individuals aged more than 40 years is sufficient to exclude the diagnosis of ADPKD.

   Nevertheless, the criteria have lower sensitivity for patients with *PKD2* mutations and also likely for patients with *PKD1* nontruncating variants or variants in other genes causing mild ADPKD (Pei et al., 2009) (more about genetics of ADPKD later).

2. Identification of causal mutation in corresponding genes.

Treatment

ADPKD is an incurable disease, nevertheless with the medical advances, quality of life and life span have improved in patients with ADPKD. So far, treatment of ADPKD focuses on slowing of disease progression and management of renal and extrarenal comorbidities, including pain. Patients are advised to follow healthy lifestyle with regular low-impact exercise, and diet with moderate caloric intake, lower sodium intake, increased water intake and restricted protein intake (Chapman et al., 2015). Early blood pressure detection and strict control is important for preservation of kidney function and prevention of cardiovascular disease. Generally, an angiotensin-converting enzyme (ACE) inhibitors or an angiotensin receptor blockers (ARB) are used for the high blood pressure treatment (Rahbari-Oskoui and Chapman, 2013).

In 2012, large clinical study TEMPO 3:4 with 1445 patients (multicenter, double-blind, placebo-controlled, 3-year trial) showed that vasopressin V2-receptor antagonist

tolvaptan (Jynarque® (USA); Jinarc® (EU, Canada); Samsca® (Japan)) inhibits cyst growth (2.8% per year in patients on tolvaptan, versus 5.5% per year in the placebo group) and slows the decline of kidney function (Torres et al., 2012). Jinarc was approved by European Medicines Agency (EMA) in May 2015 (Agency product number: EMEA/H/C/002788), by the U.S. Food and Drug Administration (FDA) in April 2018 (Application Number: 204441). The therapeutic indication of tolvaptan defined by EMA is "adults with CKD stage 1 to 3 at initiation of treatment with evidence of rapidly progressing disease". Based on this indication, ERA-EDTA (European Renal Association - European Dialysis and Transplant Association) published recommendations for use of tolvaptan taking into account the patient's age, total kidney volume, estimated glomerular filtration, genetic background etc. (for detailed algorithm see Gansevoort et al., 2016). On September 2019, the joint statement by Czech Society of Nephrology and General Medical Insurance Company of the Czech Republic indicated the approval of use of tolvaptan for Czech patients with ADPKD from 18 to 50 years of age depending on the age of the patient and his/her renal function.

Genetics

In most cases, ADPKD is caused by mutations in 2 genes: *PKD1* (16p13.3) discovered in 1985 (Reeders et al., 1985) with its refined localization described in 1995 (Hughes et al., 1995) and *PKD2* (4q22.1) discovered by two independent research groups in 1993 (Kimberling et al., 1993; Peters et al., 1993). It is estimated the *PKD1* mutations cause about 80% of ADPKD cases, whereas *PKD2* mutations are responsible for about 15% of cases (Chapman et al., 2003). About 5 to 10% of patients harbor mutations in other loci or remain genetically unresolved. Over the last few years, number of genes has been described whose mutations can cause ADPKD or ADPKD-like phenotype. The gene causing mild phenotype of polycystic kidney disease and mild to severe phenotype of polycystic liver disease was described in 2016 and called *GANAB* (11q12.3) (Porath et al., 2016). It encodes protein neutral alpha-glucosidase AB involved in the maturation and cellular localization of polycystin-1 (PC1) and polycystin-2 (PC2) (Porath et al., 2016). In 2018, the gene *DNAJB11* (3q27.3) was described (Cornec-Le Gall et al., 2018). *DNAJB11* encodes a protein acting as a co-factor of endoplasmic reticulum chaperone BiP which regulates protein folding, trafficking and degradation. It has been shown that *DNAJB11* plays an important role in proper maturation and trafficking of PC1 (Cornec-

Le Gall et al., 2018). Mutations in this gene are associated with phenotype of non-enlarged polycystic kidneys with chronic interstitial fibrosis resulting in renal failure in the sixth decade of life. One of the genes causing ADPKD-like phenotype is *HNF1B* (17q12, previously called *TCF2*) encoding protein hepatocyte nuclear factor 1-beta that has a role as a transcription factor regulating expression of PKD-associated genes, such as *PKHD1* (Hiesberger et al., 2004) and *PKD2* (Gresh et al., 2004). Mutations in this gene are, among others, associated with phenotype of renal cysts and maturity-onset diabetes mellitus (MODY), generally known as renal cysts and diabetes syndrome (RCAD).

Moreover, polycystic kidney disease can result from mutations in genes that are primarily associated with formation of autosomal dominant polycystic liver disease (ADPLD). Five genes: *PRKCSH* (19p13.2, encoding regulatory subunit of glucosidase II), *SEC61B* (9q22.33, encoding beta subunit of channel-forming translocon complex SEC61), *SEC63* (6q21, encoding protein that facilitates targeting of proteins into the *SEC61* translocon complex), *ALG8* (11q14.1, encoding alpha-1,3-glucosyltransferase) and *GANAB* (mentioned above) all encode proteins located in endoplasmic reticulum and required for efficient PC1 maturation and trafficking to the plasma membrane (Besse et al., 2017). In addition, the *LRP5* gene (11q13.2, encoding coreceptor in canonical Wnt signaling pathway) has been described in patients with ADPLD with or without renal cysts (Besse et al., 2017). Overall, mutations in these genes cause phenotype of mild to severe polycystic liver disease, accompanied by absent to mild form of polycystic kidney disease in some patients.

The phenotype of ADPKD can be also mimicked by mutations of the *PKHD1* gene (6p12.3-p12.2) primarily causing autosomal recessive form of polycystic kidney disease (ARPKD). Adult carriers of one *PKHD1* mutation can present with hepatorenal abnormalities including multiple liver cysts and increased renal medullary echogenicity (Gunay-Aygun et al., 2011).

Phenotype resembling ADPKD can arise from mutations in number of other genes. These are rare cases, but sometimes one must consider to broaden the differential diagnosis to include other cystic kidney diseases, especially in patients with atypical phenotype and no causal mutation in ADPKD genes found. Table 1 represents known kidney diseases that can (in some cases) resemble ADPKD and should be included in the differential diagnosis of ADPKD.

Disease	Genes	Inheritance	Distinctive clinical features
Simple renal cysts	N/A	Acquired	Normal-sized kidneys with normal function, cysts can increase in number and size with age
Acquired cystic kidney disease	N/A	Acquired	Common in patients with CKD or ESRD; normal- or small-sized kidneys, no extrarenal cysts
Renal cysts and diabetes syndrome	*HNF1B*	AD	Renal malformation, diabetes mellitus, hypomagnesemia, genital tract abnormalities, hyperuricemia, elevated liver enzymes
Polycystic liver disease	*PRKCSH* *SEC61B* *SEC63* *ALG8* *LRP5*	AD	Predominantly liver cystic disease, small number of renal cysts
ARPKD	*PKHD1*	AR	Neonatal or infantile enlarged polycystic kidneys, pulmonary hypoplasia, biliary duct anomalies (congenital hepatic fibrosis, intrahepatic bile duct dilatation), portal hypertension, cholangitis
Tuberous sclerosis complex (TSC)	*TSC1* *TSC2*	AD	Skin lesions (angiofibromas, hypomelanotic macules), seizures and developmental delay, renal angiomyolipomas, benign hamartomas (retina, cardiac), pulmonary lymphangioleiomyomatosis
PKD1-TSC2 contiguous gene syndrome	*PKD1* + *TSC2*	AD	Severe ADPKD at early age, renal angiomyolipomas developing from age of 1
Autosomal dominant tubulointerstitial kidney disease	*UMOD* *MUC1* *REN*	AD	Slowly progressive kidney disease, medullary cysts, small to normal sized kidneys, hyperuricemia, UMOD: early gout, REN: mild hypertension, anemia
Von Hippel-Lindau syndrome	*VHL*	AD	Renal cell carcinomas, CNS and retinal hemangioblastomas, pancreatic cysts, pancreatic endocrine tumors, pheochromocytoma
Orofaciodigital syndrome I	*OFD1*	XLD	Embryonic male lethality, cleft palate, bifid tongue, hyperplastic frenula, hypertelorism, broadened nasal ridge, digital abnormalities including syndactyly, CNS malformations, small, uniform renal cysts, normal or enlarged kidneys

Table 1: Differential diagnosis of ADPKD. Table is based on the guidelines presented in 2015 by global organization KDIGO (Kidney Disease: Improving Global Outcomes) (Chapman et al., 2015) and articles by Alves et al., 2015 and Simms, 2016.

***PKD1* – gene and its protein**

The *PKD1* gene was first described in 1994 (The European Polycystic Kidney Disease Consortium, 1994) by The European Polycystic Kidney Disease Consortium. It is located on 16p13.3 and consists of 46 exons that span over 52 kb of genomic region (Hughes et al., 1995; The International Polycystic Kidney Disease Consortium, 1995). A large part of the 5' terminal region of the *PKD1* gene (exons 1-33) is duplicated six times forming 6 pseudogenes (*PKD1P1* – *PKD1P6*) in the proximity of master gene on 16.p13.1 (Bogdanova et al., 2001; Kirsch et al., 2008). All pseudogenes share high homology (about 97.7%) with *PKD1* which complicated the full characterization of the gene in the 1990s (Hughes et al., 1995) and nowadays also complicates molecular genetic analysis of *PKD1*. *PKD1* encodes large membrane protein polycystin-1 (PC1), 4,303 amino acids long, with 11 transmembrane domains, large extracellular domain and small intracellular carboxy-terminal tail (Figure 2).

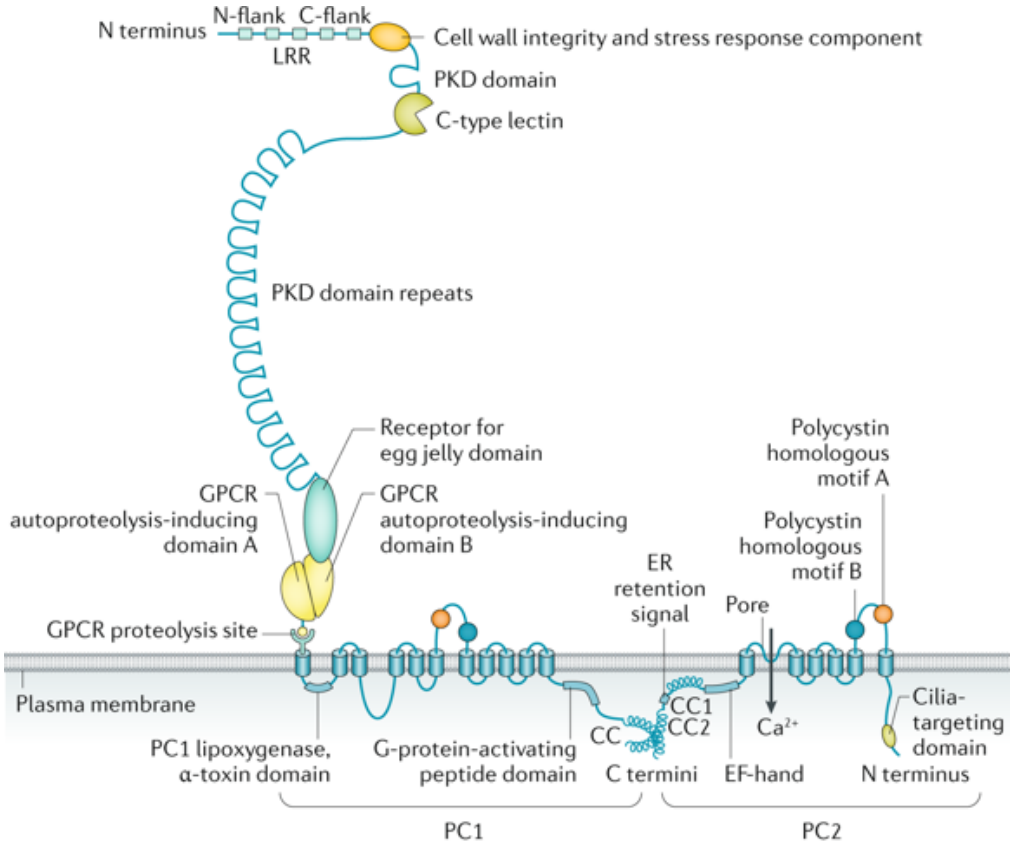


Figure 2: Structure of polycystin-1 and polycystin-2. From Bergmann et al., 2018.

PKD1 expression is observed in both, fetal and mature kidneys, and is developmentally regulated. In developing nephron, *PKD1* expression is restricted to the epithelial cells of comma- and S-shape bodies and distal tip of ureteric bud (Figure 3: schematic representation of nephrogenesis) (Peters et al., 1999; Ward et al., 1996).

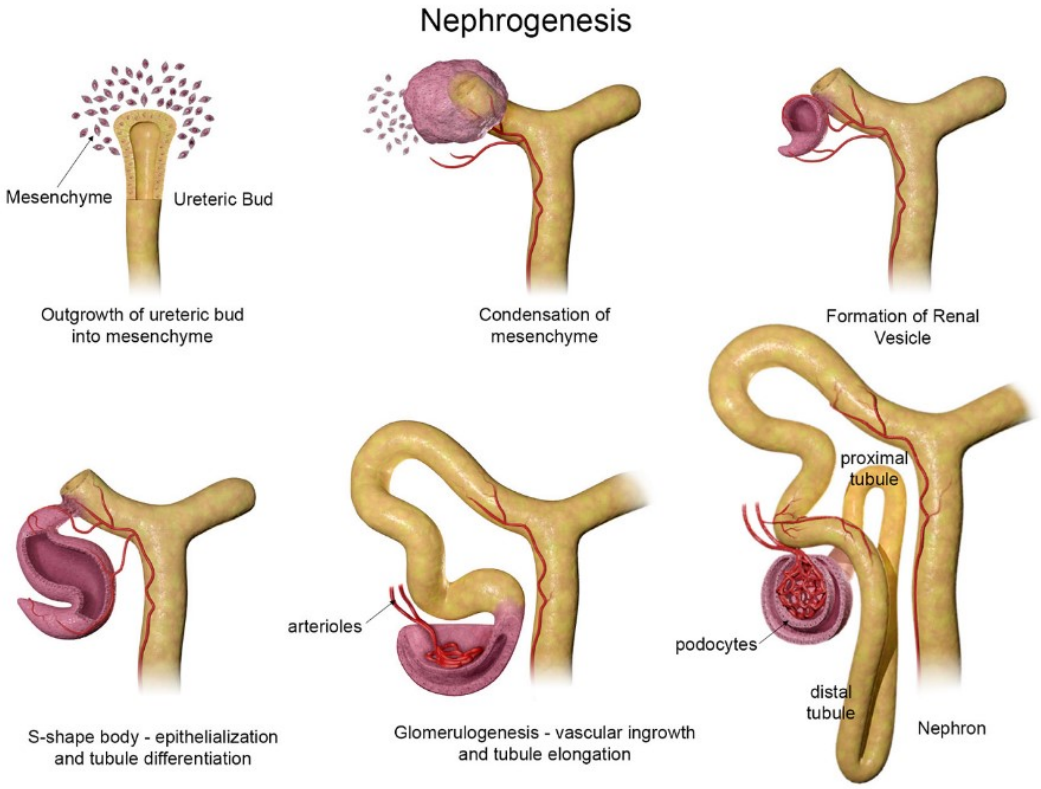


Figure 3: Development of nephron. From Seely, 2017.

In later stages of kidney development, the expression persists in Bowman's capsule and can be also found in cortical regions of tubules. In adult kidneys, the levels of expression are lower than in fetal renal tissue but still detectable (Chauvet et al., 2002). The strongest expression is in the Bowman's capsule and cortical regions of tubules (especially part of the proximal tubule adjacent to the Bowman's capsule) and also in medullary regions of tubules (loop of Henle and collecting duct) (Figure 4: scheme of mature nephron) (Ward et al., 1996).

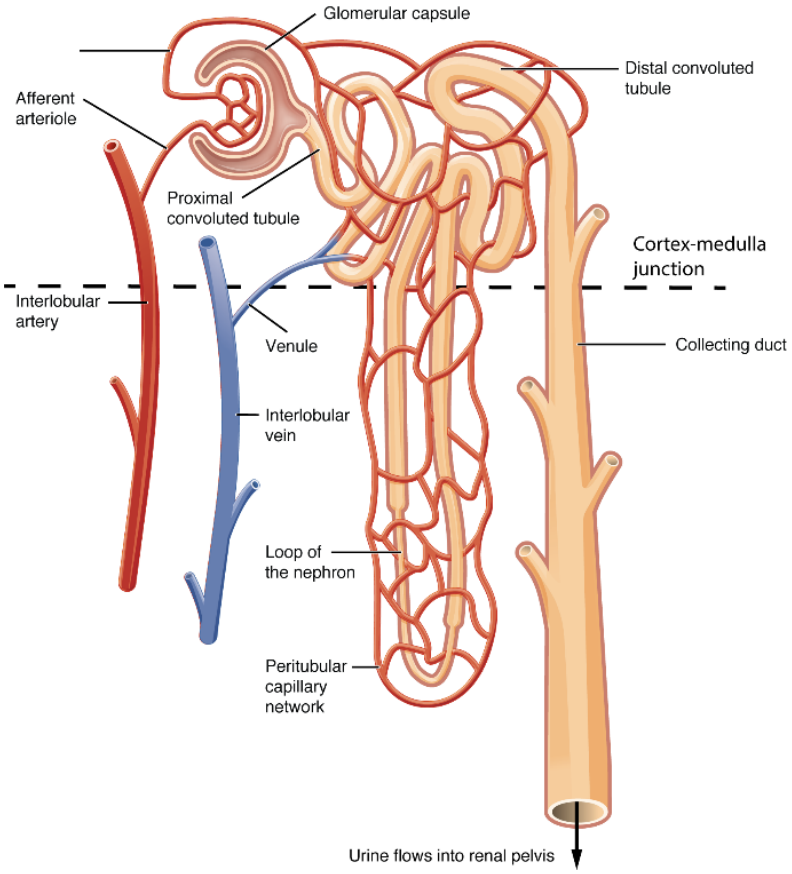


Figure 4: Schematic representation of mature nephron. From Biga et al., 2020

PKD1 expression can be also found in the epithelial cells of many organs and tissues, such as pancreas, liver, lung, brain, bowel, heart and vessels, reproductive organs, placenta, etc., especially in epithelial lining of their excretory ducts (bile ducts, pancreatic ducts, prostate glands etc.). *PKD1* expression was also noted in nonepithelial cells of vascular smooth muscle, skeletal muscle or myocardial cells (Ong et al., 1999; Peters et al., 1999). The extend of *PKD1* expression is in accordance with systemic nature of polycystic kidney disease affecting multiple organs.

On the cellular level, PC1 can be found in the plasmatic membrane at the sites of contact between adjacent cells (at the free boarders of the cell, no PC1 can be found) and it was also found mildly present at the perinuclear region of cytoplasm (Peters et al., 1999). It was also showed that in presence of polycystin-2 (PC2), PC1 co-localizes with PC2 to the endoplasmic reticulum (Grimm et al., 2003). The presence of PC1 was also described in a cilium – non-motile antenna-like organelle protruding from the apical surface of nearly every cell type in human body (Yoder et al., 2002). Primary cilia have broad range of function ranging from sensing mechanical (e.g. fluid flow) and chemical (e.g. light)

changes of environment to transduction of these signals to complex intracellular signaling pathways (Hoey et al., 2012). Moreover, PC1 as well as PC2 are shed into the urinary exosome-like vesicles that can interact with primary cilia (Hogan et al., 2009).

The localization of PC1 in cell adhesion sites is consistent with proposed function of PC1 in cell-cell and cell-matrix interactions. Also, the motifs present at the N-terminal (extracellular) end of PC1 hints its role in focal adhesions of cells. The leucine-rich repeats (LRR) (see Figure 2) of PC1 modulates its binding to several components of extracellular matrix (ECM) (collagen I, fibronectin, laminin) (Malhas et al., 2002). LRRs mediate correct adhesion of the cell to ECM or another cell and thus have suppressive effect on cell proliferation (Malhas et al., 2002). It is therefore possible the mutations of *PKD1* could affect these interactions causing abnormal proliferation as seen in ADPKD. The two LRRs including their distinctive flanking region on their both sides are encoded by exons 1-4 of *PKD1* gene (Hughes et al., 1995). Cell wall integrity and stress response component motif is a putative domain with unknown function, nevertheless, it was suggested it could play a role in interaction to carbohydrates of ECM, possibly regulating stress-induced pathways as seen in *S. Cerevisiae* with similar domain (Weston et al., 2003). Another motif found in the extracellular end of PC1 is C-type lectin domain (encoded by exon 6 and 7). It was shown that this domain binds to carbohydrates and proteins (collagens) of ECM. This interaction is highly increased in presence of calcium ions (Weston et al., 2001). Exons 5 and 11-15 encode sixteen domains of PC1 called PKD repeats. These domains are involved in calcium-dependent homophilic interactions and it was shown that antibodies against PKD domains cause disruption of cell-cell adhesions in Madin-Darby canine kidney (MDCK) cells (Ibraghimov-Beskrovnaya et al., 2000). Thus, it seems the PKD domains play important role in intercellular adhesion.

Another domain of PC1 is called Receptor for egg jelly domain (REJ). It was originally described in sea urchins, specifically in a membrane glycoprotein located in sperm that acts as a receptor for glycoproteins in egg jelly (extracellular matrix of sea urchin eggs) (Moy et al., 1996). It was suggested the REJ domain in PC1 somehow modulates ion transport (as seen in sea urchin during the sperm acrosome reaction) (Moy et al., 1996), presumably Ca^{2+} influx (Ponting et al., 1999).

The domain alongside REJ domain is called G-protein coupled receptor proteolytic site (GPS) domain and contains proteolytic site generating large N-terminal fragment of 3,048 amino acids and C-terminal fragment of 1,254 amino acids (Qian et al., 2002). The

cleavage is executed rapidly after the synthesis of PC1 in most – but not all – of the PC1 molecules and is therefore probably subjected to dynamic regulation. Most of N-terminal fragments remain non-covalently tethered to the C-terminal fragment, small fraction is secreted into the medium (Figure 5). It seems the cleavage is essential for normal biological function of PC1 as mutations introduced in adjacent REJ domain that is essential for cleavage cause disruption of PC1 cleavage and consequently formation of cyst-like structures in MDCK cells (Qian et al., 2002).

Another alleged function of extracellular part of PC1 is mechanosensation – concretely fluid flow and pressure sensation – in primary cilia. It is suggested the fluid flow in renal tubules causes conformational changes of PC1 located at the plasma membrane of cilia that result in opening of the cation channel PC2, the interacting partner of PC1 (more about PC2 later), and subsequent calcium entry into the primary cilium (the sensory organelle of the cell). The higher levels of calcium ions in cytoplasm may then module various intracellular signaling pathways (Patel and Honoré, 2010). Nevertheless, this hypothesis is yet to be proven *in vivo* and is still subjected to research with inconclusive results. The hypothesis of PC1/PC2 complexes in mechanosensation in primary cilia is reviewed in Patel and Honoré, 2010.

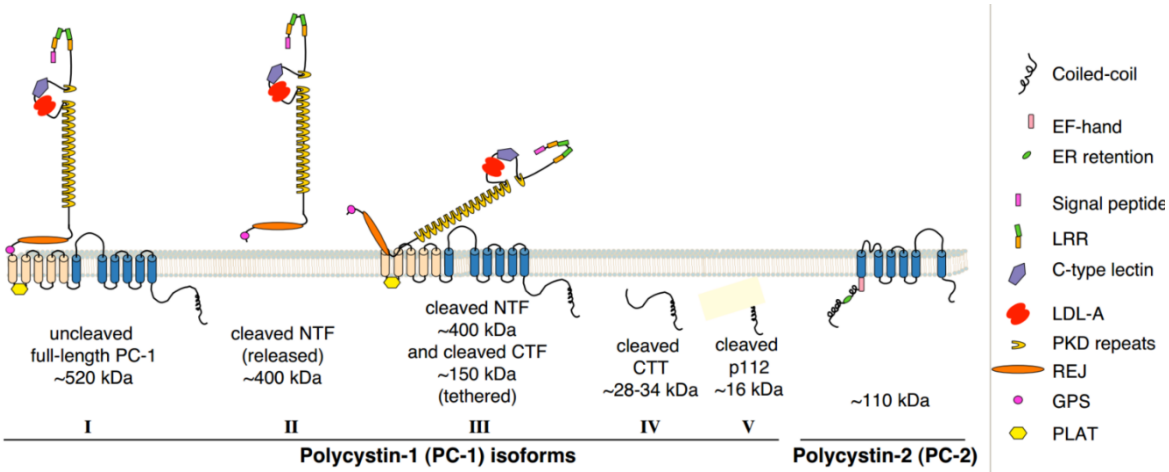


Figure 5: Sites of proteolytic cleavage in PC1 and PC2. From Boletta, 2009.

While the extracellular part of PC1 is the place of cell-cell and cell-matrix interactions and possible site of mechanosensation, the short intracellular C-terminal domain plays role in regulation of diverse signaling pathways and is a place of PC1 and PC2 interaction. In 2004 (Chauvet et al., 2004) and 2006 (Low et al., 2006), respectively, the

two sites of cleavage localized in C-terminal site of PC1 were observed (Figure 5). Chauvet et al. (Chauvet et al., 2004) suggested the proteolytic cleavage is induces by mechanical stimuli and releases C-terminal tail (CTT) of about 34kDa (which corresponds to approximately 200-226 of PC1 cytosolic tail). The cleaved fragment contains highly conserved 21-residue motif (positions 4,134–4,154) of nuclear localization site (NLS) that enables the fragment to enter nucleus where it directly modulates gene expression. It was suggested that polycystin-2 impairs the transfer of CTT to nucleus and thus modulates the downstream effects of PC1 on its target genes.

The second reported C-terminal cleavage site is located at a distal site of PC1 cytosolic domain and produces approximately 17kDa fragment that translocates to the nucleus and stimulates STAT6-dependent transcription by binding to transcription factor STAT6 and the coactivator P100 (Low et al., 2006). STAT6 localizes to primary cilia of renal epithelial cells and it was shown that decrease of apical fluid flow causes translocation of STAT6 to nucleus. It was also proved the cyst-lining cells in ADPKD exhibit elevated levels of nuclear PC1 tail, STAT6 and P100 (Low et al., 2006).

The C-terminal tail of PC1 is also the site of interaction of PC1 and PC2 (Polycystin-2). The interaction is mediated through coiled-coil domains located in C-terminal tails of both proteins (see Figure 2) (Casuscelli et al., 2009).

PKD2 – gene and its protein

The *PKD2* gene is located on chromosome 4q22.1 (Kimberling et al., 1993; Peters et al., 1993). The gene and its possible protein product was firstly described in 1996 (Mochizuki et al., 1996) and its exon structure year later (Hayashi et al., 1997). It is formed by 15 exons and encodes 968 amino-acids long transmembrane protein with six transmembrane domains and intracellular N- and C-termini (Mochizuki et al., 1996) (Figure 2). The expression of PC2 is developmentally regulated with the highest expression levels in mature kidneys. The embryonic expression of PC2 is almost barely detectable until 17 weeks of gestation and gradually ascending from 22 to 30 weeks (Foggensteiner et al., 2000). By 30 weeks of gestation the pattern of PC2 expression is essentially the same as seen in adult kidney with highest levels of distribution in medullary thick ascending limbs of the loop of Henle and cortical distal tubules (Foggensteiner et al., 2000). The extrarenal expression of polycystin-2 parallels that of polycystin-1 and is described in epithelial cells of many tissues as well as vascular

smooth muscle, skeletal muscle and myocardial cells (Ong et al., 1999). Subcellularly, PC2 resides in several compartments. The highest levels of PC2 can be found in endoplasmic reticulum (ER) (Cai et al., 1999), nevertheless complexes of PC1/PC2 were also found at the plasma membrane. So it seems, PC1 can recruit PC2 to the plasma membrane where they co-assembly and produce functional complex (Hanaoka et al., 2000). The presence of PC2 was also described in renal primary cilia (Yoder et al., 2002), mitotic spindle in dividing cells (Rundle et al., 2004) and exosomes (Hogan et al., 2009). The subcellular localization of PC2 is established and regulated by specific signal sequences and associated proteins. The specific regulation pathways are reviewed in Chapin and Caplan, 2010. Nonetheless, despite numerous experiments, subcellular localization of PC2 is still under debate. Especially results regarding presence of PC2 in plasma membrane are ambiguous and the hypothesis is raised that PC2 can be only located on plasma membrane in the primary cilia (for comprehensive review see Douguet et al., 2019).

Polycystin-2 (TRPP2) belongs to the group of transient receptor potential (TRP) channels and like most of the TRP channels forms homo- or hetero-tetramers. It also shares their architecture with 6 transmembrane (TM) domains and intracellular N- and C-termini (Figure 2 and 6). The first four TM domains (S1-S4) form a voltage-sensor-like domain (VSLD) followed by pore domain consisting of two TM helices (S5, S6) separated by a pore helices (PH1, PH2) and the selectivity filter (Grieben et al., 2017). Unlike other TRP channels, PC2 contains ‘tetragonal opening for polycystins’ (TOP) domain located between transmembrane domains S1 and S2 (Grieben et al., 2017). The TOP domain faces ER lumen (in PC2 located in ER), extracellular surface (in PC2 located in plasma membrane or cilia) and in kidney epithelial cells it resides in renal tubule lumen (Grieben et al., 2017). It was suggested the TOP domain plays important role in PC2 structure stabilization and influences channel activity through its direct contact with pore domain of its own protein and VSLD of adjacent chain in tetrameric structure. Hence, genetic variants disrupting TOP-domain structure lead to loss of channel activity of PC2 and PC1/PC2 complex trafficking (for more details see Grieben et al., 2017).

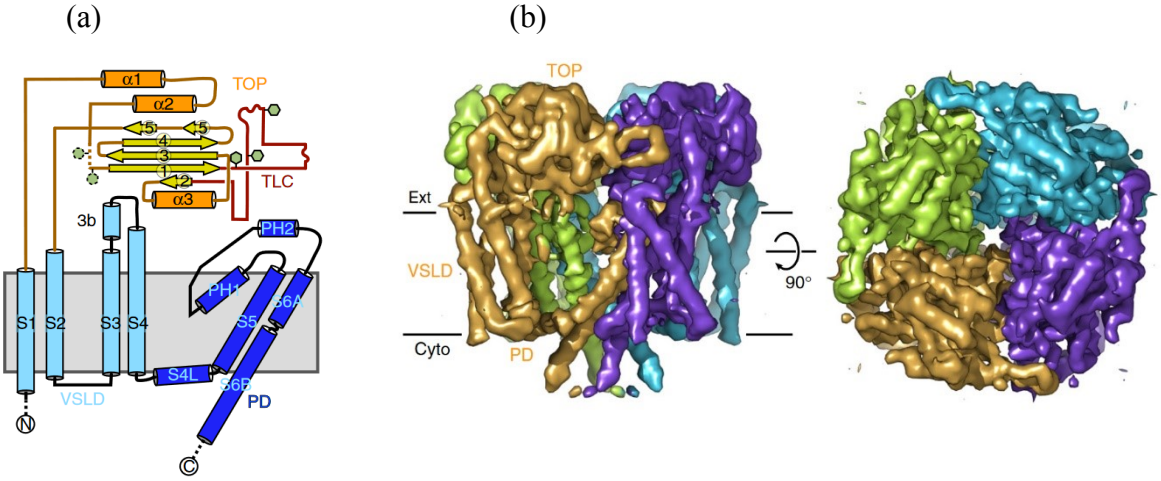


Figure 6: (a) Structure of Polycystin-2. (b) Reconstruction of $PC2^{P185–D723}$ at 4.2-Å resolution in tetrameric organization, colored by monomer. Cyto: cytoplasmic; Ext: luminal or extracellular; PD: pore domain. Adapted from Grieben et al., 2017.

The C-terminus of PC2 contains several domains with different function. The domain containing two EF-hands motifs plays an essential role as a sensor of calcium concentration required for channel activity due to its ability to bind the Ca^{2+} ions from the channel vicinity (Gifford et al., 2007; Li et al., 2009). Another domain, called 'ER retention signal' domain is necessary and sufficient for retention of PC2 in endoplasmic reticulum which is mediated by proteins PACS-1 and PACS-2 (Cai et al., 1999; Köttgen and Walz, 2005). PACS-2 is capable of localization of PC2 to endoplasmic reticulum, while PACS-1 mediates its retrieval from trans-Golgi network. The binding of PACS proteins to PC2 requires phosphorylation of PC2 (on Ser812) by protein kinase CK2 (Köttgen and Walz, 2005). The coiled-coil domain located at the very end of C-terminus is the place of PC2-PC1 binding.

The N-terminus of PC2 contains conserved motif (R6VxP) that is required for trafficking of PC2 to cilia. This trafficking is independent on PC1 as proven by several experiments with Pkd1–/– mice (Li et al., 2009).

Polycystin-2 functions as a nonselective channel with a high permeability to Ca^{2+} that can be regulated by diverse stimuli, such as ions (Gonzalez-Perrett et al., 2001), pH, voltage, phosphorylation, membrane stretch and interactions with other proteins (González-Perrett et al., 2002; Grieben et al., 2017). It was shown that increased levels of intracellular calcium activate polycysin-2-mediated release of Ca^{2+} from endoplasmic reticulum (Koulen et al., 2002). This function was also described at plasma membrane of renal epithelial cells, where PC2 contributes to entry of calcium (or other) cations into the cell (Luo et al., 2003).

There are various reports that PC2 indirectly regulates calcium levels by its interaction with other calcium channels, such as ryanodine receptor in a heart (González-Perrett et al., 2002) or the inositol 1,4,5-trisphosphate receptor (Li et al., 2009) and also associates with some TRP channels (Köttgen et al., 2008; Tsiokas et al., 1999).

Polycystins and signaling pathways

PC1 and PC2 modulate number of signaling pathways in cooperation with many other proteins (the list of proteins interacting with PC2 and PC1 and the putative functions of these interactions are reviewed in Torres and Harris, 2009). Subcellularly, both proteins can be found at plasma membrane of primary cilium and endoplasmic reticulum and it seems their interaction can affect both their localization and functional properties. However, results regarding their exact function, cooperation and in vivo localization are still ambiguous with sometimes disputable results (Delmas et al., 2004; Grimm et al., 2003; Hanaoka et al., 2000). The advance towards understanding of polycystin complex was made in 2018 when the assembly of PC1/PC2 complex obtained by cryo–electron microscopy with 3.6-Å resolution was presented by Su et al. (Su et al., 2018) (Figure 7). They found the PC1/PC2 complex is formed by one PC1 and three PC2 molecules. Nonetheless, more research will be needed to understand the function of the complex as it seems from their primary results that due to 3 positively charged, cavity-facing residues of PC1 protein, cation permeation of the channel may be blocked.

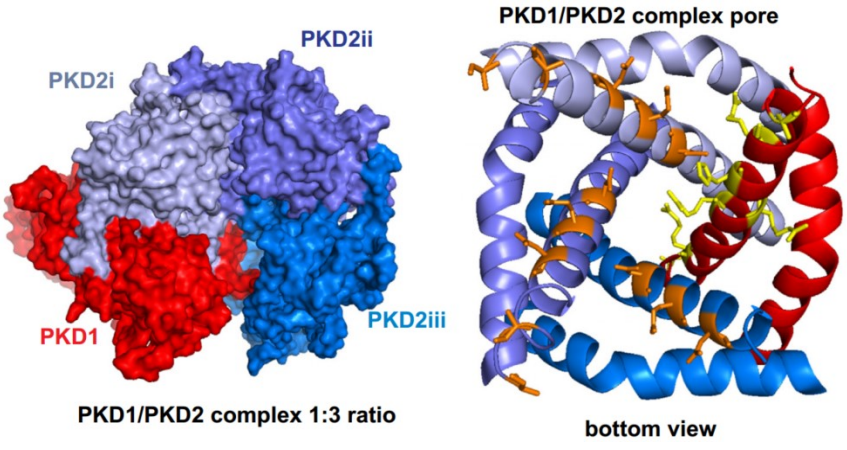


Figure 7: Visualization of the Su et al. (Su et al., 2018) cryo-EM structure of the PC1/PC2 channel complex. From Woodward and Watnick, 2019.

The typical manifestation of polycystic kidney disease is formation of renal cysts. Cysts are fluid-filled outgrowths of renal epithelium that arise only in about 1% to 3% of nephrons and eventually detach from the original tissue (Terryn et al., 2011). Although clinically detectable in adulthood, kidney cysts exist in utero and grow at a stable rate (Clark et al., 2017). The evidence suggests the cyst development includes numerous cellular changes. In a nutshell, the major characteristics of cystogenesis are epithelial cell proliferation, abnormal fluid secretion and increased accumulation of extracellular matrix.

One of the typical feature of ADPKD cells is altered apical-basal polarity and planar cell polarity which causes misoriented division of cells with rather tubule expansion than elongation and formation of cysts (Nigro et al., 2015). Another feature of cyst-lining cells are elevated cAMP levels. It was shown that cAMP stimulates cell proliferation (by activating downstream signaling pathways) as well as fluid secretion (involving cystic fibrosis transmembrane conductance regulator - CFTR) causing cyst growth (Hanaoka and Guggino, 2000). In concordance with these findings, the treatment strategies lowering levels of cAMP cause slowing of cyst growth. The proliferation of epithelial cells is also caused by increased levels of epidermal growth factor receptor and its mislocalization to the apical cell membrane (Du and Wilson, 1995) and altered signaling through numerous pathways. Another feature of cystic tissue is increased extracellular matrix production, recruitment of inflammatory cells and abnormal cell-cell junctions (Bergmann et al., 2018). Although the molecular mechanisms of cyst formation and growth is not fully understood, the role of polycystins was described in a vast network of signaling pathways affecting cell growth and division:

1. The TSC-mTOR pathway

In 2009, the negative effect of PC1 on TSC-mTOR pathway (tuberous sclerosis complex - mammalian target of rapamycin) was described (Boletta, 2009). TSC complex consisting of proteins TSC1 (also called hamartin) and TSC2 (also called tuberin) plays a role as a negative regulator of mTOR kinase, as it acts as a GTPase-activating protein for the GTP-binding protein Rheb (Ras homolog enriched in brain), which must be in GTP-bound state to activate mTOR kinase. Activated mTORC1 phosphorylates ribosomal protein S6 kinase beta-1 (S6K1) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and thus stimulates the cell proliferation (Wullschleger et al., 2006).

Carboxy-terminal tail (CTT) of PC1 directly interacts with TSC2 and protects it from phosphorylation by Akt (protein kinase B) causing its retention at plasma membrane and thus allowing TSC2 to remain bound to TSC1 in functional complex (Dere et al., 2010; Ma et al., 2005).

In 2001, the study by Kleymenova et al. (Kleymenova et al., 2001; Saxton and Sabatini, 2017) demonstrated that relation between PC1 and TSC2 is bidirectional. They showed that in rat renal tuberin-deficient cells, intracellular trafficking of polycystin-1 was disrupted, resulting in sequestration of PC1 within the Golgi apparatus. So, it seems PC1 needs TSC2 for its localization at plasma membrane.

2. PI3K/Akt pathway

C-terminus of PC2 interacts with IP_3 receptor (IP_3R) on endoplasmic reticulum and can prolong IP_3-dependent calcium release (Li et al., 2005b). On the contrary, PC1 has an ability to inhibit this calcium release as it weakens the interaction between PC2 and IP_3R (Li et al., 2009; Santoso et al., 2011). It was showed that in the cell with PC1 expression the PI3K/Akt (Phosphatidylinositol-4,5-bisphosphate 3-kinase/Protein kinase B) pathway is activated (Santoso et al., 2011). In this pathway, activated receptors located at plasma membrane directly stimulate PI3K triggering conversion of phosphatidylinositol-3,4-bis-phosphate (PIP2) lipids to phosphatidylinositol-3,4,5-tris-phosphate (PIP3). Protein kinase B (Akt) binds to PIP3 at the plasma membrane which allows PDK1 (3-phosphoinositide dependent protein kinase 1) to access and partially activate Akt by its phosphorylation (Hemmings and Restuccia, 2012). Fully activated Akt (for example by subsequent mTOR phosphorylation) can act on numerous downstream substrates mediating cellular functions such as apoptosis, growth, proliferation, metabolism, angiogenesis, survival, protein synthesis and transcription (Hemmings and Restuccia, 2012). Experiments showed that activation of PI3K/Akt pathway causes reduction of the PC2-IP_3R interaction and decrease in level of intracellular calcium. Moreover, it seems PC2 is subsequently recruited to the plasma membrane where it forms complex with PC1 serving as an influx calcium channel (Santoso et al., 2011). The involvement of PC1 in PI3K/Akt pathway was described in MDCK cells, where it was found the PC1 activates PI3K (probably through G-protein activation) triggering activation of downstream effectors of the pathway capable of inducing resistance to apoptosis and tubulogenesis (Boca et al., 2006).

3. The JAK-STAT pathway

The role of polycystins in cell cycle progression is described several times. One of the pathways modulated by both polycystins is JAK-STAT pathway (The Janus kinase-signal transducers and activators of transcription). PC1 binds and activates JAK2 kinase that subsequently phosphorylates STAT proteins (STAT1 and 3) (Bhunia et al., 2002). Phosphorylated STAT1 can form homodimers and translocate to the nucleus to bind p21 gene promoter causing upregulation of p21 expression. Increased levels of p21 then lead to the cell cycle arrest in G0/G1 stage of cell cycle (Bhunia et al., 2002). The experiments showed that this process requires PC2 as a cofactor as mutations that disrupt PC1-PC2 binding prevent pathway activation (Bhunia et al., 2002).

Another member of STAT protein family activated by PC1 is STAT6 (Low et al., 2006). After the cleavage of C-terminal tail of PC1, newly formed fragment translocates to nucleus and activates STAT6-dependent transcription. The exact function of STAT6 activation is yet to be elucidated nevertheless high levels of activated STAT6 in cyst-lining cells in two different PKD mouse models have been reported (Weimbs et al., 2013).

4. The Id pathway

Another pathway related to cell division is the Id pathway. Id2 (Inhibitor of DNA binding 2) is a member of the helix-loop-helix family of proteins that lack DNA-binding domain, but are capable of binding to positively acting transcription factors and preventing them from association with DNA (Weimbs et al., 2013). The ones of the binding partners for Id proteins are E protein family members that function as transcription factors regulating cell cycle, e.g. by activation of expression as such proteins as p21 (Weimbs et al., 2013). PC2 can directly bind to Id2 and sequester it in the cytoplasm. Id2 is therefore unable to bind E protein transcription factors that can function as transcription activators. Moreover, the PC2–Id2 interaction is regulated by PC1-dependent serine phosphorylation of PC2 (Li et al., 2005a).

5. G-protein signaling pathway

In 1998 (Arnould et al., 1998), it was suggested the cytosolic domain of PC1 transactivates transcription factor AP-1 (activating protein-1) that is known to control different cellular processes, such as cell differentiation, proliferation and apoptosis (Karin et al., 1997). The pathway starts by activation of heterotrimeric G-proteins by C-

terminal tail of PC1 containing G-protein-activating domain. The activated Gα subunits then positively regulates the activity of c-Jun N-terminal kinase (JNK, member of MAPK kinases) that phosphorylates AP-1 transcription factor. In addition, JNK/AP-1 activation is also triggered by protein kinase C (PKC). Experiments on human embryonic kidney cells 293T also showed that PC2 upregulated AP-1-dependent transcription and that the co-expression of PC2 with the interacting C terminus of PKD1 dramatically augmented PKD2-mediated AP-1 activation (Arnould et al., 1999). Even though the exact mechanisms of the relevant pathways are to be elucidated, the experiments by Le et al. showed that indeed AP-1 activity is increased in vivo in human and mouse polycystic kidney disease (Le et al., 2005).

Another pathway comprising G-proteins is calcineurin/NFAT signaling pathway. NFATs (nuclear factor of activated T-cells) are the family of transcription factors that regulate expression of genes controlling cell development and adaptation in a wide range of cell types (Horsley and Pavlath, 2002). The data suggest that PC1 activation of trimeric G-protein causes Gα-dependent activation of phospholipase C (PLC) followed by release of Ca^{2+} from intracellular stores and thus activation of calcineurin, a Ca^{2+}-dependent phosphatase. Thanks to the dephosphorylation of NFAT by activated calcineurin, NFAT can translocate into the nucleus and modulate transcription of its target genes (Puri et al., 2004).

Both families of AP-1 and NFAT transcription factors are known to synergistically regulate gene expression of diverse genes with composite DNA elements containing adjacent NFAT and AP-1 binding sites (Macián et al., 2001). This balanced activation is well described in numerous genes required in the productive immune response but its possible effect on signaling pathways in other cells is yet to be proved.

6. Wnt signaling pathway

Wnt proteins belong to the family of secreted growth factors playing roles in signaling pathways controlling large variety of biological processes during embryonic development (e.g. proliferation, differentiation, cellular polarity) and self-renewal in a number of mature tissues (Steinhart and Angers, 2018). Wnt signaling can function through different pathways: canonical (β-catenin-dependent) or noncanonical (β-catenin-independent).

In general, in canonical Wnt pathway, secreted glycoproteins Wnts bind to Frizzled receptor (containing co-receptor LRP – prolow-density lipoprotein receptor-related

protein). Activated Frizzled/LRP receptor can recruit cytoplasmatic protein Dishevelled to the membrane providing a docking site for Axin and GSK-3 (glycogen synthase kinase 3) (Clevers, 2006). Axin is a member of a destruction complex (with several other proteins) that targets β-catenin for ubiquitination. In case of activation of canonical Wnt pathway, Axin is recruited off the destruction complex and to the Frizzled/LRP receptor which prevents degradation of β-catenin. Therefore, the levels of β-catenin rise and stabilized β-catenin can, together with TCF (T cell factor), promote transcription of Wnt target genes (Clevers, 2006). Carboxy-terminal tail of PC1 can affect canonical Wnt pathway as it inhibits the ability of β-catenin to activate TCF-dependent transcription by inhibiting the affinity of interaction between β-catenin and TCF protein (Lal et al., 2008). Indeed, DNA microarray analysis revealed the canonical Wnt pathway is activated in cystic tissue of ADPKD patients. So it seems PC1 could modulate developmental processes and cystogenesis (Lal et al., 2008).

Noncanonical Wnt pathway plays role in determination of cellular polarity and motility and is required during tissue formation and homeostasis (Sugimura and Li, 2010). As in the canonical Wnt pathway, the noncanonical type also starts with activation of Frizzled receptor by Wnt ligands (except that the role of LRP remains unknown in noncanonical pathway). The signal is then transformed into two downstream paths. The activation of Frizzled causes that Disheveled is recruited to the plasma membrane, where it binds to the small GTPases Rac and Rho that subsequently cause the activation of JNK (Sugimura and Li, 2010). On the other hand, the activation of Frizzled causes G-protein-dependent activation of phospholipase C leading to intracellular release of calcium initiating relay of several downstream pathways (Sugimura and Li, 2010). Even though there is no evidence that polycystins can regulate the noncanonical Wnt pathways, the pathway is probably very important in the formation of cystic kidneys, as defectively oriented cell division can be seen in cystogenesis (Lancaster and Gleeson, 2010).

Dosage model of cystogenesis and disease severity

ADPKD patients show considerable intrafamilial and interfamilial phenotypic variation ranging from adult patients with mild manifestation without renal failure to patients with findings *in utero*. The severity of disease is modulated by genetic factors together with environmental and stochastic factors.

The focal character of cyst formation where only minority of nephrons is affected is explained by dosage model of cystogenesis (Figure 8). Patients harbor one germline mutation in *PKD1* or *PKD2* gene, but another event, such as somatic inactivation of second allele by another mutation or loss of heterozygosity is needed for formation of cyst (Qian et al., 1996; Watnick et al., 2000). The fall of functional protein levels under critical threshold causes cyst formation and is dependent on type of mutation (inactivating vs hypomorphic alleles) and their unique combination (Hopp et al., 2012; Leonhard et al., 2015). Moreover, other modifying factors, such as variants in other genes as well as environmental and stochastic factors including acute kidney damage can influence cyst formation and growth.

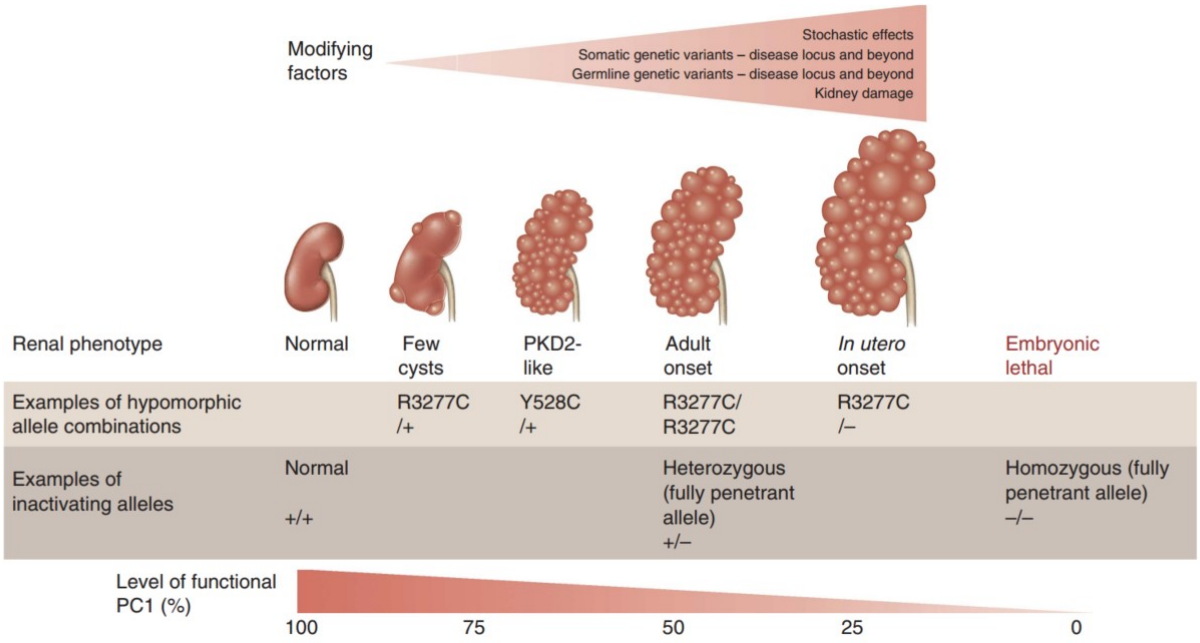


Figure 8: Dosage model of cystogenesis in ADPKD.
The fully penetrant allele (typically inactivating mutation) causes 50% drop of levels of functional PC1. The cyst is formed when level of functional PC1 falls under critical threshold. This may occur by another somatic mutation on the second allele and is modulated by other modifying factors. Different types of hypomorphic (incompletely penetrant) alleles can cause variable phenotype - p.Y528C has a mild renal phenotype similar to *PKD2* (Pei et al., 2012), whereas p.R3277C can result in mild, adult-onset, or early-onset disease depending on which allele is present *in trans* (Hopp et al., 2012). The likelihood of cyst development may be also influenced by additional variants at the disease locus and elsewhere (somatic and germline) along with environmental and stochastic factors. From Ong and Harris, 2015.

The severity of renal phenotype in patient is affected by several factors. As mentioned before, one of the factors affecting disease manifestation and progression is type of mutation. The French study of 741 patients with ADPKD showed significant differences in patients' phenotypes dependent on associated mutation (Cornec-Le Gall et al., 2013). Mutations in *PKD1* gene caused more severe form of ADPKD with a median age of ESRD at 58 years compared to 79 years of ESRD in patients with *PKD2* mutations (Figure 9). Moreover, the median age at onset of ESRD was 55 years for carriers of a *PKD1* truncating mutation and 67 years for carriers of a nontruncating *PKD1* mutation.

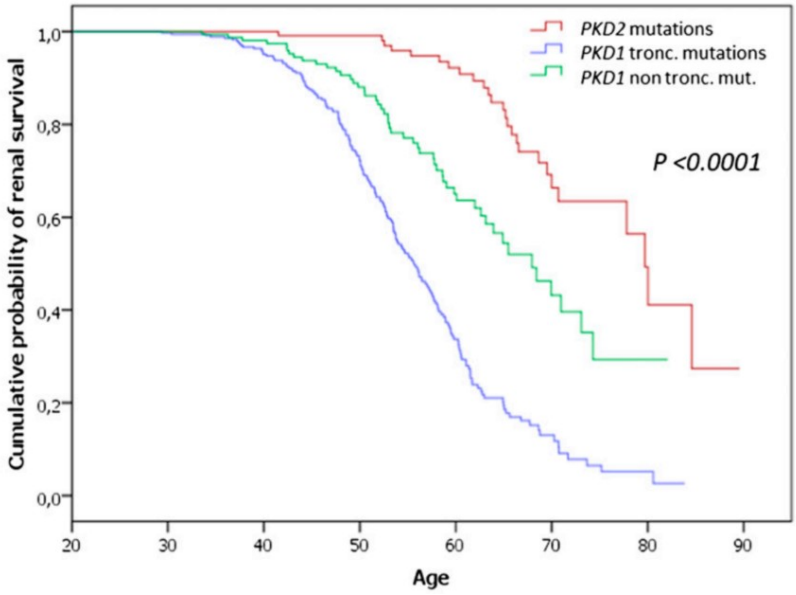


Figure 9: Genotype – phenotype correlations in patients with ADPKD. Significant differences in renal survival between patients with *PKD1* truncating mutation, *PKD1* nontruncating mutation or *PKD2* mutation. From Cornec-Le Gall et al., 2013.

Although majority of ADPKD patients manifest with adult-onset PKD, about 1% of patients exhibit very early onset (VEO) disease with a diagnosis made in utero or during infancy. The inactivation of both alleles in gene *PKD1* or *PKD2* is thought to cause embryonic lethality in patients as it was described in mice (Lu et al., 1997, 2001). Nonetheless, the presence of one hypomorphic allele in patients with biallelic mutations causes postnatal survival of patients with phenotype dependent on type of the pathogenic variants (Hopp et al., 2012; Reiterová et al., 2013). In rare cases, the mutations in multiple PKD genes were described in patients with severe form of ADPKD. In these families, combination of mutations in two genes inherited from both parents elucidated more severe phenotype of the child compared to his/her parents (Bergmann et al., 2011;

Reiterova et al., 2018). The exacerbated PKD phenotype may be also caused by combination of mutations in PKD genes with other ciliary genes, and was already described in families harboring one variant in ADPKD gene and one in *HNF1β* (Bergmann et al., 2011). These findings are supported by studies comparing phenotypes of affected siblings and monozygotic twins suggesting that other modifier genes may contribute to the final phenotype (Persu et al., 2004).

About 10-15% of families with ADPKD have no family history of the disease (Bergmann et al., 2018). The large study of 210 patients with ADPKD without family history showed this can be caused due to *de novo* disease, germline or somatic mosaicism, presence of hypomorphic alleles or missing parental medical records (Iliuta et al., 2017).

The disease prognosis can also differentiate depending on the sex of patient. Men have substantially worst renal disease, however women had larger liver cyst volumes, especially after multiple pregnancies and/or use of hormonal replacement therapy (Chapman, 2003; Gabow et al., 1990b; Heyer et al., 2016).

The new prognostic score system (PROPKD) developed for prediction of renal survival in ADPKD patients includes genetic and clinical variables with different scoring values:

- Being male 1 point
- Hypertension before 35 years of age 2 points
- First urologic event before 35 years of age 2 points
- *PKD2* mutation 0 points
- Nontruncating *PKD1* mutation 2 points
- Truncating *PKD1* mutation 4 points

Three risk categories with corresponding median ages for ESRD onset were defined as low risk, ESRD: 70.6 years (0–3 points), intermediate risk, ESRD: 56.9 years (4–6 points), and high risk, ESRD: 49 years (7–9 points).

In conclusion, the manifestation and progress of ADPKD is determined by numerous factors, including type of genetic variants, modifying genes, sex, environmental factors etc., nevertheless careful clinical and genetic evaluation may enable accurate disease prognosis and personalized disease management.

Autosomal recessive polycystic kidney disease

Autosomal recessive polycystic kidney disease (ARPKD) is a rare inherited kidney disorder with typical clinical presentation *in utero* or in early childhood. ARPKD is characterized by dilatations of renal collecting ducts causing enlarged kidneys, and ductal plate malformation of the liver resulting in congenital hepatic fibrosis. Formerly, the diagnosis of cystic kidneys was treated as same entity regarding only the degree of renal destruction. Nonetheless, in 1902 it was noted by Ernst Küster, the age distribution of 239 affected subjects had two peaks: one at birth (59 stillborns) and another between ages 30-60 years (Figure 10) (Lundin and Olow, 1961). Over the years, the recessive heredity of a cystic kidney disease occurring in children was suspected and proved in 1961 by Lundin and Olow (Lundin and Olow, 1961). Moreover, it was noted that cystic kidney disease of children is always associated with specific hepatic lesions (Blyth and Ockenden, 1971).

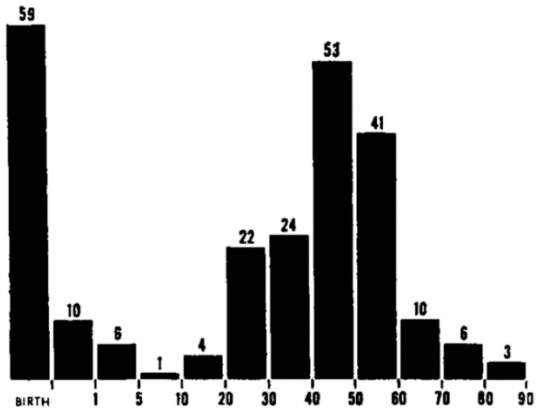


Figure 10: Age distribution of 239 patients with cystic kidneys in study by Ernst Küster. From Lundin and Olow, 1961.

Epidemiology

The data regarding disease incidence are not as extensive as for ADPKD because of a relative rareness of ARPKD. Nevertheless, consensus incidence in non-isolated population is estimated to be 1:20,000 with corresponding carrier frequency of 1:70 (Zerres et al., 1998a). These estimations were confirmed by large centralized study from the United States collecting data from 2010 to 2014 with final estimated incidence counting approximately 1:26,485 live births (Alzarka et al., 2017). In isolated or inbred populations the incidence of ARPKD may be much higher which was indeed reported for

Finnish population with an estimated incidence of about 1:8,000 births (Alzarka et al., 2017). The distribution of ARPKD between sexes is relatively uniform, but when it comes to ethnicity the prominent distribution is reported for Caucasians (Alzarka et al., 2017; Guay-Woodford and Desmond, 2003).

Mortality during perinatal period is high reaching 30-40% and is related to respiratory insufficiency caused by pulmonary hypoplasia (Alzarka et al., 2017; Guay-Woodford and Desmond, 2003; Zerres et al., 1998b). Thus, the mechanical ventilation during neonatal period is a strong negative predictor of long-term survival (Guay-Woodford and Desmond, 2003).

Clinical manifestation

ARPKD is a disorder with high phenotypical variability dependent on the age of presentation. Majority of patients are identified *in utero* or at birth with sonographic findings of bilaterally enlarged echogenic kidneys with poor corticomedullary differentiation (Erger et al., 2017; Guay-Woodford, 2015). The first ultrasound findings of ARPKD are usually made around 21st to 24th weeks of gestation and does not include hepatic changes that are not detected until late childhood (Avni et al., 2012). In severe cases, oligohydramnios with Potter sequence consisting of massively enlarged kidneys, pulmonary hypoplasia with typical facies and spine and limb abnormalities can be present (Bergmann, 2015). About 30 to 50% of affected newborns die shortly after birth from respiratory distress caused by pulmonary hypoplasia and/or restrictive lung disease caused by massively enlarged kidneys (Alzarka et al., 2017; Guay-Woodford and Desmond, 2003; Zerres et al., 1998b).

Children who survive the perinatal stage are more prone to experience complications associated with renal and hepatic impairment. The renal phenotype comprises formation of cysts that (unlike ADPKD cysts) arise by dilatations of renal collecting ducts (Figure 11). In the early stages of ARPKD, cysts tend to be smaller and localized in the medulla, but with advanced clinical course cysts may enlarge and vary in appearance, and thus make the kidney phenotype indistinguishable from ADPKD (Avni et al., 2002). Most of the patients develop end-stage renal disease, but the age of onset is highly dependent on initial presentation. The study of 164 patients with ARPKD showed renal survival rate (end point defined as start of dialysis, renal transplantation or death due to ESRD) of 86% at 5 years, 71% at 10 years, 66% at 15 years, and 42% at 20 years (Bergmann et al.,

2005a). Another study reported renal survival rate of 75% at 11 years in patients diagnosed in perinatal period, and 75% at age 32 years in the group of patients with ARPKD detected after one month of age (Guay-Woodford, 2015).

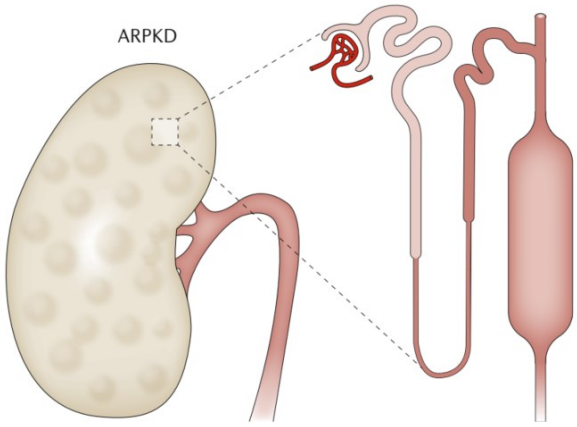


Figure 11: Dilatation of collecting duct in ARPKD kidney. From Bergmann et al., 2018.

Within the first months of life, up to 80% of children develop arterial hypertension that requires strict control and multidrug treatment (Bergmann et al., 2005a). Another common symptom – occurring in 6-26% of children – is hyponatremia (low concentration of sodium in the blood) probably caused by impaired urinary dilution rather than sodium wasting (Guay-Woodford et al., 2014; Zerres et al., 1996). About 30-43% of patients suffer with urinary tract infections and about 25% of children have growth retardation that correlates with impaired renal function (Zerres et al., 1998b). In older children, renal calcifications have also been reported to be common (Guay-Woodford, 2015).

ARPKD is invariably connected with hepatic abnormalities caused by despaired ductal plate malformation resulting in congenital hepatic fibrosis and fusiform dilatation of bile ducts (Figure 12) (Turkbey et al., 2009). Congenital hepatic fibrosis is not usually detected on routine prenatal sonographic screening and rather dominates the phenotype in the later stages of ARPKD (Erger et al., 2017). Some adolescents and adults with ARPKD can even manifest only symptoms connected to hepatic impairment (Bergmann et al., 2018). The most common clinical manifestation of the liver disease is portal hypertension with possible comorbidities, such as hypersplenism with cytopenia, splenomegaly and esophageal varices (Shneider and Magid, 2005). The complication

related to the bile duct disease may include episodes of cholangitis or sepsis and/or complications of cholelithiasis or biliary sludge (Shneider and Magid, 2005). Since the standard liver biochemical testing is typically normal in ARPKD children with liver disease, careful physical examination and blood counts tests are recommended (Shneider and Magid, 2005). The most life threatening complications related to liver impairment in ARPKD patients are sepsis (especially after renal transplantation), bleeding from esophageal varices and complications related to cholangiocarcinoma that may develop with slightly increased risk in adults >40 years of life (Srinath and Shneider, 2012).

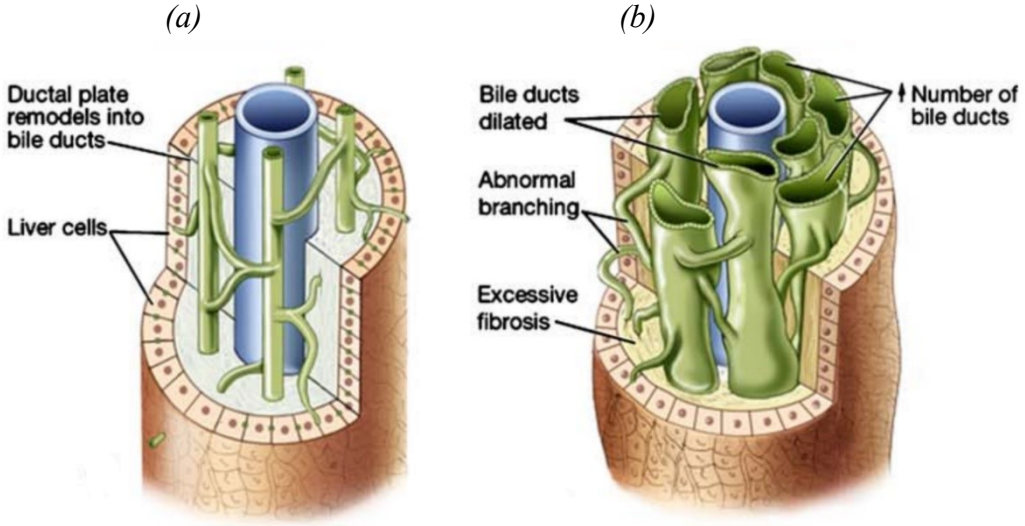


Figure 12: Biliary ductal plate: (a) normal (b) dilated bile ducts with abnormal branching and fibrosis. From Turkbey et al., 2009.

Diagnosis of ARPKD

The establishment of diagnosis of ARPKD depends on the age of proband. Prenatal diagnosis of ARPKD by ultrasound (US) detection is usually done at later stages of pregnancy – during second-trimester US. And although US findings are usually sufficient to suggest the diagnosis, it can be too late for possible termination of pregnancy. So at present, the only reliable and early prenatal testing is molecular analysis of corresponding genes (Guay-Woodford et al., 2014). Prenatal US findings show bilaterally enlarged hyperechogenic kidneys with poor corticomedullary differentiation and oligohydramnios (biliary findings are not evident until the postnatal infancy). However, special attention should be taken to extrarenal findings as number of other

conditions are associated with prenatal findings of hyperechogenic kidneys (Table 2) (Guay-Woodford et al., 2014).

Postnatally, US imaging with findings of bilaterally large echogenic kidneys with poor corticomedullary differentiations and congenital hepatic fibrosis is usually sufficient for ARPKD diagnosis. The US findings of proband's parents should show no traces of cystic kidney changes. Also, the genetic analysis may facilitate the diagnosis and finding of two pathogenic mutations in corresponding genes is sufficient for establishment of diagnosis (Guay-Woodford et al., 2014).

Treatment

Currently, there is no cure for ARPKD, and treatment only helps to manage the associated symptoms of the disease. In cases of severe fetal ARPKD, the breathing difficulties caused by pulmonary hypoplasia should be considered and delivery at a facility with a level IV neonatal intensive care unit should be planned (Guay-Woodford et al., 2014). Most of the children develop hypertension within the first months of life, so the early detection and treatment is needed to avoid related comorbidities. So far, angiotensin converting enzyme inhibitors or angiotensin receptor blockers are used for blood pressure control. General recommendation for reducing the high blood pressure also includes healthy lifestyle with balanced diet and exercise. Common symptom in ARPKD children is hyponatremia. When present, fluid intake should be minimized without compromising nutrition (Guay-Woodford et al., 2014). The care should be also taken in management of difficulties in feeding and poor growth within the first years of age, and – in later age – in management of liver disease and its comorbidities (annual complete blood and platelet count and abdominal US at 5 years of age is recommended) (Guay-Woodford et al., 2014). With the disease progression, renal replacement therapy including dialysis or kidney transplantation can be needed.

Genetics

ARPKD is caused by mutations in gene called *PKHD1* (PKHD1 ciliary IPT domain containing fibrocystin/polyductin; formerly: Polycystic kidney and hepatic disease 1) discovered in 2002 by two research groups (Onuchic et al., 2002; Ward et al., 2002). *PKHD1* is an extensive gene covering over 469 kb and located on the short arm of chromosome 6 (6p12.3-p12.2) (Onuchic et al., 2002). In 2017, second gene causing

ARPKD was described (Lu et al., 2017). It is called *DZIP1L* (DAZ interacting zinc finger protein 1 like) and is localized on the long arm of chromosome 3. Homozygous mutations of *DZIP1L* were described in four families with ARPKD. DZIP1L can be found at the transition zone of cilia where interacts with protein SEPT2 (Lu et al., 2017). Little is known about its function, nevertheless studies showed that with aberrant function of DZIP1L, PC1 and PC2 show altered distribution along the ciliary membrane. That would be in line with the finding that binding partner of DZIP1L, protein SEPT2, is a component of the transition zone barrier of cilium (Lu et al., 2017).

Although the typical form of ARPKD is caused by mutations in the *PKHD1* gene, several disorders that can, in some cases, phenotypically resemble ARPKD are described. Especially in the early stage of the disease, the clinical findings can be very indistinct and reliable differential diagnostic is needed. Due to the existence of a number of ARPKD phenocopies and with advances in next-generation sequencing techniques, mutational analysis of panel of genes is recommended to enable effective diagnosis (Table 2) (Guay-Woodford et al., 2014).

Disease	Genes	Inheritance	Distinctive clinical features
ADPKD	*PKD1* *PKD2*	AD	Bilateral macrocysts, cysts in other organs (e.g., the liver, seminal vesicles, pancreas, and arachnoid membrane), non-cystic abnormalities (e.g., intracranial aneurysms and dolichoectasias, dilatation of the aortic root and dissection of the thoracic aorta, mitral valve prolapse, colonic diverticula, abdominal wall hernias)
Nephronophthisis	*NPHP1-NPHP?*	AR (AD)	Tubulointerstitial cysts and small or normal size kidneys, defects in urinary concentration, anemia, polyuria, and polydipsia
Renal cysts and diabetes syndrome	*HNF1B*	AD	Renal cysts, single kidney, renal hypoplasia/dysplasia, genital malformations, autism, epilepsy, gout, hypomagnesemia, hyperthyroidism, liver and intestinal abnormalities, and a rare form of kidney cancer
Joubert syndrome and related disorders	*JBTS1-JBTS38*	AR (AD)	Mental retardation and ataxia due to hypoplasia of the cerebellar vermis, retinal coloboma, irregular breathing pattern, “molar- tooth sign”

Bardet-Biedl syndrome	*BBS1-BBS21*	AR (DR)	Renal malformation, obesity, hypogonadism, retinal degeneration, polydactyly, mental retardation, and renal malformations, hearing loss, diabetes mellitus, and other metabolic defects
Meckel syndrome	*MKS1-MKS15*	AR	Occipital meningoencephalocele, microphthalmia, lung hypoplasia, PKD or renal hypo/dysplasia, bile-duct dilatation, postaxial polydactyly, and situs inversus
Orofaciodigital syndrome I	*OFD1*	XLD	Embryonic male lethality, cleft palate, bifid tongue, hyperplastic frenula, hypertelorism, broadened nasal ridge, digital abnormalities including syndactyly, CNS malformations, small, uniform renal cysts, normal or enlarged kidneys
Jeune syndrome (Asphyxiating thoracic dystrophy)	*IFT80 DYNC2H1 WDR19 TTC21B*	AR (DR)	Narrowed thorax, respiratory insufficiency, recurrent respiratory infections, short stature with limb shortening, brachydactyly, polydactyly, renal and hepatic impairment, eye abnormalities
Renal-hepatic-pancreatic dysplasia	*NPHP3 NEK8*	AR	Dysplasia of kidneys, liver, and pancreas, heart defects
Zellweger syndrome (Peroxisome biogenesis disorder)	*PEX1-26*	AR	Dysmorphic features, severe psychomotor retardation, profound hypotonia, seizures, ocular abnormalities, hepatomegaly, renal cysts and chondrodysplasia punctate, diffuse encephalopathy, retinopathy or cataract, and sensorineural hearing loss

Table 2: The list of diseases that can phenotypically resemble ARPKD. The table is based on the consensus expert recommendations developed in May 2013 by an international team of 25 multidisciplinary specialists and published by The Journal of Pediatrics in 2014 (Guay-Woodford et al., 2014). The distinctive clinical features are based on Sweeney and Avner, 2019, Bergmann, 2018, Hildebrandt et al., 2011, Poyner and Bradshaw, 2013, Rajagopalan et al., 2016 and Wanders et al., 1995.

About 1-2% of ADPKD patients shows early signs of the disease that presents under the age of fifteen, sometimes with perinatal morbidity and mortality. These cases can be in some patients clinically undistinguishable from ARPKD (Bergmann, 2012). Moreover, the severe form of ADPKD is not only confined to *PKD1* mutations, as children with *PKD2* mutations and ADPKD with early manifestation were described (Bergmann et al.,

2008; Fencl et al., 2009). ADPKD with very early manifestation can also arise in patients with combination of mutations in several PKD genes, which is in line with dosage theory of PKD (Bergmann et al., 2011; Reiterova et al., 2018). High intrafamilial variability also suggests a role of modifying factors on disease manifestation. In conclusion, the total number of ARPKD patients equals the number of ADPKD patients among children with PKD in departments of pediatric nephrology (Bergmann, 2012).

Nephronophthisis (NPHP) is an autosomal recessive disorder that can lead to ESRD during childhood or adolescence. NPHP represents the most frequent inherited cause of kidney failure in pediatric patients (Broyer et al., 1986). It can be caused by mutations in number of genes (so far 25 *NPHP* genes have been described (Srivastava et al., 2018)), but majority of patients harbor homozygous deletion of the *NPHP1* gene (Konrad et al., 1996). *NPHP* genes are pleiotropic and can cause broad spectrum of phenotypes – from manifestations limited to the kidneys to complex syndromes with extrarenal involvement comprising liver, pancreas, central nervous system, eyes and bones – such as Senior-Loken syndrome (with retinal degeneration), Joubert syndrome (with cerebellar vermis aplasia), Meckel syndrome, Jeune syndrome, and others (Bergmann, 2015; König et al., 2017).

Joubert syndrome was first described by Marie Joubert in 1969 (reprint: Joubert et al., 1999). The clinical manifestations comprise irregular breathing pattern during neonatal period, hypotonia, ataxia, mental retardation, abnormal eye movements and several additional symptoms (Joubert et al., 1999). The hallmark feature of Joubert syndrome is ‘molar tooth sign’ that can be recognized on brain imaging studies and results from the aplasia of the cerebellar vermis and other abnormalities in brain development (Hildebrandt et al., 2011; Joubert et al., 1999).

Meckel syndrome is a severe autosomal recessive disorder with high perinatal mortality and multisystem involvement (Salonen and Paavola, 1998). The main features include bilaterally enlarged kidneys with cystic dysplasia, occipital encephalocele, polydactyly and liver fibrosis (Salonen and Paavola, 1998). The patients can also present with additional manifestations in connection to developmental abnormalities of urinary and genital system, bones, heart and eyes (Khaddour et al., 2007). Meckel syndrome is genetically heterologous with numerous described genes with pleiotropic effect.

Bardet-Biedl syndrome (BBS) is typically described as an autosomal recessive disorder, nevertheless triallelic model of disease transmission was also described (Katsanis et al., 2001). The patient’s phenotype evolves during the first decade of life and involves many

parts of the body (Schachat and Maumenee, 1982). The major feature of BBS is gradual loss of vision, starting with night blindness and progressing to loss of central and color vision (Forsythe and Beales, 2013). The clinical phenotype also includes obesity, polydactyly, hypogonadism, developmental delay and cognitive deficit, abnormalities of heart and gastrointestinal systems, and renal structural abnormalities recognized as a major cause of mortality in BBS (Gimpel et al., 2019).

Orofaciodigital syndrome 1 (OFD1) belongs to the heterogeneous group of syndromes characterized by malformations of oral cavity, face and digits – oral-facial-digital syndromes. It is transmitted as an X-linked dominant trait with embryonic male lethality (Gurrieri et al., 2007). The gene responsible for formation of OFD1 phenotype was identified in 2001 and called *OFD1* (Ferrante et al., 2001). Unlike other forms of OFD, type I is the only one from the group that commonly shows additional phenotype of polycystic kidneys that can in some patients dominates the phenotype (Feather et al., 1997).

Jeune syndrome or asphyxiating thoracic dysplasia belongs to the large group of autosomal recessive skeletal ciliopathies called short-rib thoracic dysplasia with or without polydactyly (SRTD). It is characterized by short-limbed statue with inconstant polydactyly and constricted thoracic cage that results in the constriction of lung expansion (Poyner and Bradshaw, 2013). Severe forms of thoracic cage rigidity can lead to the prenatal or neonatal death caused by respiratory insufficiency (Huber and Cormier-Daire, 2012). The nonskeletal manifestations usually develop in patients past infancy with less severe skeletal abnormalities, and include mainly cystic renal dysplasia and liver and eye abnormalities (Huber and Cormier-Daire, 2012).

Renal-hepatic-pancreatic dysplasia is a severe autosomal recessive disease described in several children. Its manifestation usually comprise dysplasia of kidneys, liver and pancreas and cardiac abnormalities, but its expression can be variable (Neuhaus et al., 1996). Nevertheless, it usually presents prenatally with neonatal death and only few children survive the neonatal period (Rajagopalan et al., 2016).

Zellweger syndrome belongs to the large group of autosomal recessive peroxisomal disorders, specifically to the group with impaired peroxisomal biogenesis (Wanders, 2004). The patients usually die within the first year of life with features including craniofacial abnormalities, psychomotor retardation, hypotonia, renal cysts, hepatomegaly and other abnormalities, and due to their absent or weak sucking and swallowing fail to thrive (Wanders et al., 1995).

Renal cysts and diabetes syndrome (RCAD) is a disorder with manifestations comprising nondiabetic renal abnormalities, maturity-onset diabetes of the young (MODY) and/or abnormalities of genital tract. The disease is caused by mutations in dominant gene *HNF1B* (previously called *TCF2*), which was at first associated only with the origin of diabetes in young patients, discovered in 1997 (Horikawa et al., 1997). Its association with renal and genital abnormalities was described 2 years later, in 1999 (Lindner et al., 1999). *HNF1B* gene encodes transcription factor hepatocyte nuclear factor 1-beta that plays an important role during embryonic development by tissue-specific regulation of gene expression in organs, such as liver, kidney, intestine or genital organs (Coffinier et al., 1999). Its importance in kidney development explains why mutations of *HNF1B* (that count mainly *de novo* whole-gene deletions (Ulinski et al., 2006)) are between the most frequent causes of fetal hyperechogenic kidneys together with genes causing autosomal recessive and dominant PKD (Decramer et al., 2007). In the study of 62 pregnancies with prenatal findings of fetal bilateral hyperechogenic kidneys were mutations in *HNF1B* identified in 18 (29%) of them (Decramer et al., 2007). Moreover, genotype-phenotype correlation showed *HNF1B* mutations are associated with normohydramnios and two types of kidney findings: (1) bilateral, normal-sized, hyperechogenic kidneys with or without cortical microcysts or (2) unilateral, normal-sized, hyperechogenic kidney with a larger contralateral kidney with diffuse macrocysts (Decramer et al., 2007).

PKHD1 – gene and its protein

PKHD1 is an extensive gene spanning over 472 kb and containing at least 86 exons (Onuchic et al., 2002). It encodes protein fibrocystin (previously called polyductin) with the longest open reading frame (ORF) made up by 66 exons encoding protein of 4,074 amino acids (Onuchic et al., 2002). The expression of *PKHD1* was observed in developing as well as adult kidneys, with predominant expression in ureteric ducts and collecting ducts of embryonic kidneys to restricted expression in collecting ducts of adult kidneys (Ward et al., 2003). Outside the kidneys, fibrocystin was observed in mature intrahepatic bile ducts, pancreas, testes and the adrenal gland (Ward et al., 2003). Subcellularly, fibrocystin was localized at primary cilium (Ward et al., 2003), basal body (Wang et al., 2004; Zhang et al., 2004) and (together with PC1 and PC2) in urinary exosome-like vesicles that interact with primary cilia (Hogan et al., 2009). The protein was also found at centrosome and mitotic spindle in dividing cells of several cell lines

and its dysfunction is thought to be contributing to cystogenesis in ARPKD (Zhang et al., 2010).

Fibrocystin is a transmembrane protein with short intracellular C-terminus and large extracellular N-terminus (see Figure 13). Nevertheless its exact form in the cell is still discussed with some papers showing its extensive tissue- and age-specific alternative splicing (Boddu et al., 2014; Onuchic et al., 2002) and others the predominant presence of its longest form (Bakeberg et al., 2011). In addition, it seems fibrocystin can undergo site-specific proteolysis at its carboxy-terminal site (with proteolytic site near the transmembrane domain) and because of the presence of nuclear localization site (NLS) the resulting fragment is translocated to the nucleus (Hiesberger et al., 2006). The cleavage of fibrocystin is triggered by release of intracellular Ca^{2+} (Hiesberger et al., 2006) or could be part of a complex processing that has been described in primary cilia (Kaimori et al., 2007). During this processing, fibrocystin undergoes proteolytic cleavage at the extracellular domain (near transmembrane domain) producing large fragment that remains tethered to the transmembrane part by disulphide bridges. The fragment is then shed from the primary cilium under control of other proteins while C-terminal fragment is also cleaved and released to the cytoplasm (Kaimori et al., 2007). Although these processes still need to be proven they may outline the mechanism of signal distribution from cilia to downstream targets.

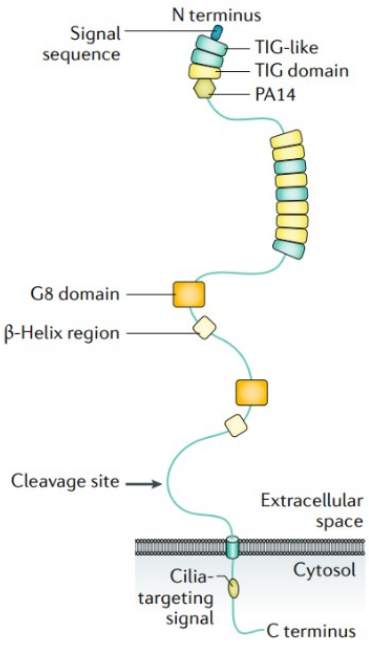


Figure 13: Schematic structure of fibrocystin/polyductin. From Bergmann et al., 2018.

The extracellular part of fibrocystin contains 7 copies of TIG/IPT domains (Ig-like, plexins, transcription factors) that are found in cell surface receptors and intracellular transcription factors, as well as 5 TIG-like domains that do not fully correspond to TIG domains criteria (Onuchic et al., 2002; Ward et al., 2002). Fibrocystin contains several β-helix sequences formed by parallel β-strands. Some of the β-strands are also identified in two domains called G8 domains and may be involved in extracellular ligand binding and catalysis (He et al., 2006). Another domain, called PA14, has a structure of a β-barrel and is predicted to function in carbohydrate binding (Rigden et al., 2004). Extracellular terminus of fibrocystin also contains 64 potential site of N-glycosylation suggesting fibrocystin might be highly glycosylated (Ward et al., 2002). In short C-terminus of fibrocystin, two sites of phosphorylation and cilium-targeting sequence can be found (Follit et al., 2010; Onuchic et al., 2002).

Regarding its function, the structure of fibrocystin suggests its possible role as a transmembrane receptor, nonetheless, its precise function remains elusive. Several studies have been made on the cell lines that are beginning to clarify the mechanisms of pathologic changes in ARPKD and the role of fibrocystin. The study on kidney samples from nephrectomies showed the AKT/mTOR pathway is activated in ARPKD cells and probably plays a significant role in ARPKD progression (Fischer et al., 2009). The study from 2019 described changed characteristics of *PKHD1* knockdown cells that are softer and more motile with increased cell invasion and decreased cell-matrix and cell-cell adhesions (Puder et al., 2019). And study of *PKHD1*-silenced cells showed decreased levels of intracellular calcium ions and abnormal proliferation (Yang et al., 2007).

The similar features of ADPKD and ARPKD always raised the question of shared localization, function or signaling pathways. It was discovered that fibrocystin is in the same complex with PC2 (Wang et al., 2007) and that they are linked together by kinesin-2 protein (Wu et al., 2006) or directly bind through the binding domain in C-terminus of fibrocystin and N-terminus of PC2 (Kim et al., 2008). It seems this interaction prevents the downregulation of PC2 by its stabilization (Kim et al., 2008).

Ciliopathies

Polycystic kidney disease and other cystic diseases, such as nephronophthisis, Bardet-Biedl syndrome, Joubert syndrome etc. are categorized in a large group of systematic disorders called ciliopathies. Their pathogenesis is related to abnormal structure or function of the primary cilia.

Primary cilium is an antenna-like organelle that was firstly described in humans by Swiss anatomist Karl Wilhelm Zimmermann (1898), who gave it the name 'central flagella' and predicted its sensory function (for detailed history of cilia discovery see Bloodgood, 2009). The non-motile primary cilia are microtubule-based organelles with '9 + 0' axoneme architecture (9 doublets of microtubules without the central doublet as seen in motile cilia) that emanates from basal body and can be present on nearly every cell in the mammalian body (Joukov and De Nicolo, 2019) (Figure 14).

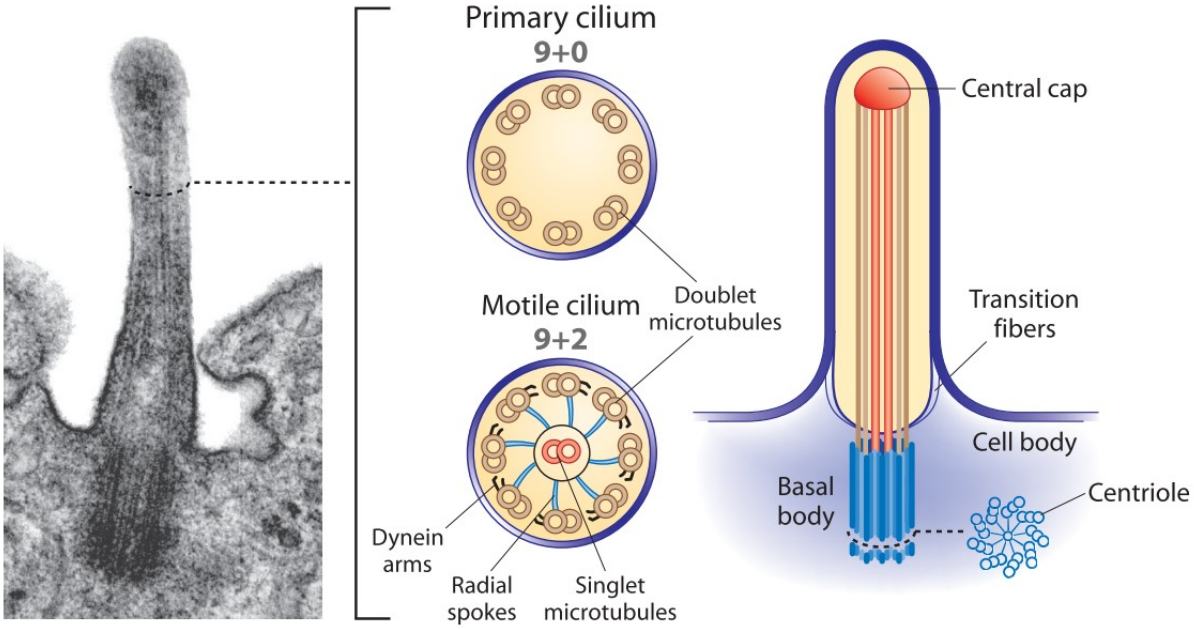


Figure 14: Structure of primary cilium. From Yoder, 2007.

Basal body is a structure composed of 9 microtubule triplets and derives from mother centriole of the centrosome (Rosenbaum and Witman, 2002). The transport of necessary components to the tip of cilium and back is provided by 'intraflagellar transport' (IFT) system that moves non-membrane bound particles along the microtubule doublets thanks to motor proteins kinesin-2 (anterograde transport) and dynein (retrograde transport) (Rosenbaum and Witman, 2002).

Cilia have sensory function, for example in mechanosensation (e.g. sensing of flow in renal epithelial cells as discussed earlier, left-right asymmetry during development, pressure, touch and vibration sensation), light detection, odor detection and play important role in development by mediation of cell-to-cell communication through sensing of external signals and transforming them into signaling pathways (e.g. Hedgehog ang Wingless) (reviewed in Berbari et al., 2009). Different disorders that are part of the group of ciliopathies are caused by mutations in genes encoding proteins playing different roles in cilia formation, maintenance, transport and signaling function (Hildebrandt et al., 2011) (summarized in Figure 15).

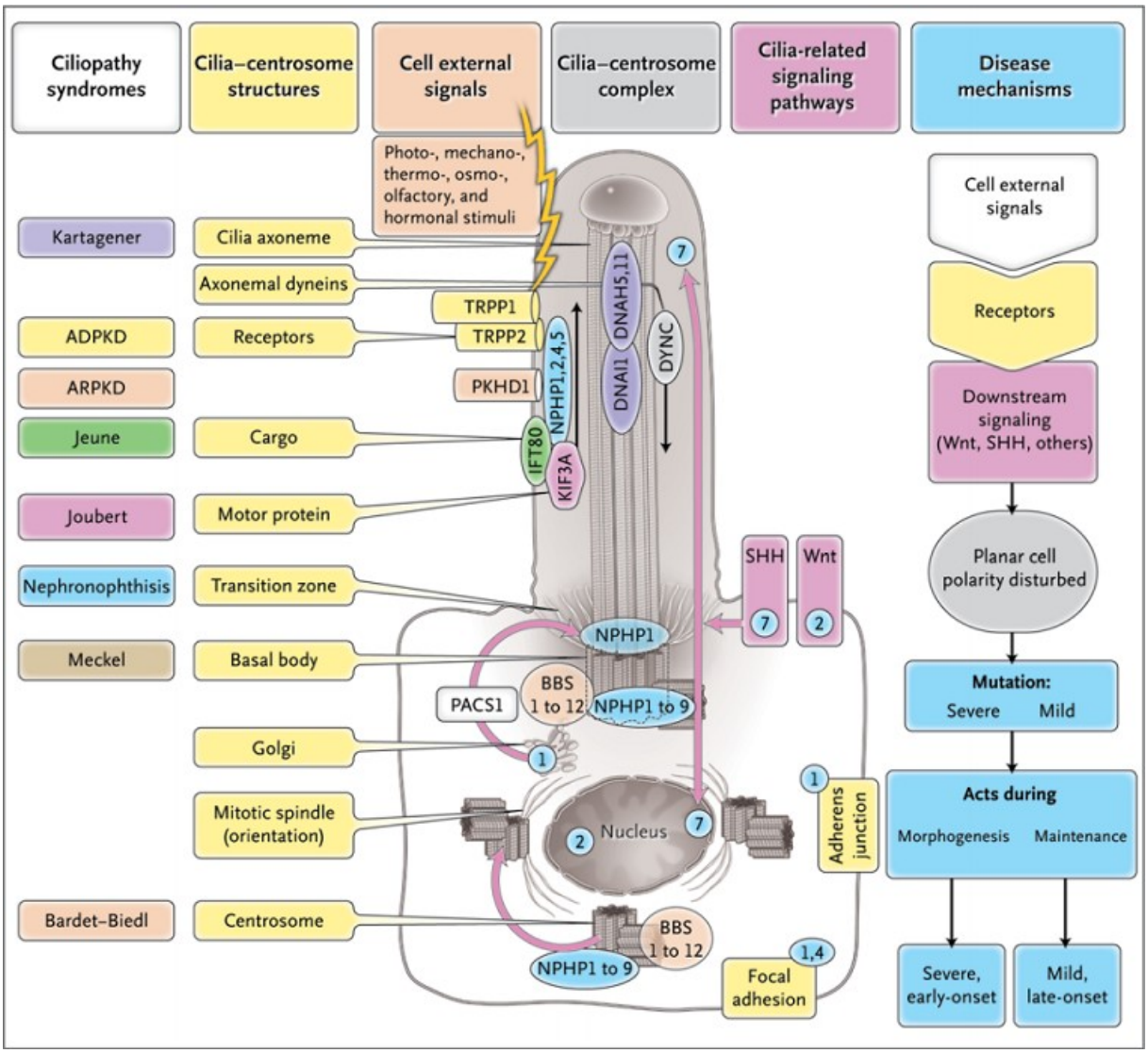


Figure 15: Proteins and their respective roles in primary cilium, plus syndromes that are associated with the aberrant function of these proteins. From Hildebrandt et al., 2011.

HYPOTHESIS AND OBJECTIVES

In the first years of our project we focused on the genetic diagnosis of autosomal recessive polycystic kidney disease. Our hypothesis was that patients with clinically suspected ARPKD carry two mutations in the *PKHD1* gene and that type of mutation could affect the patient's phenotype. Our objectives included collection of group of patients with clinical diagnosis of ARPKD and sequencing of the whole *PKHD1* gene by next generation sequencing. Gathered genetic and clinical data were then correlated, and conclusions were made regarding the influence of mutations on the final phenotype of the patient.

The project was funded by the grant project of Internal Grant Agency of the Ministry of Health of the Czech Republic (IGA MZ ČR) NT/13090-4 'Sekvenční varianty genu *PKHD1* u autozomálně recesivní polycystické choroby ledvin' starting in 2012 and ending in 2015 with final evaluation as 'A'. Most of the project results were published in 2015 (Obeidova et al., 2015).

Although in the large number of families the suspected clinical diagnosis of ARPKD was successfully proved by genetic analysis of the *PKHD1* gene, some patients remained unsolved. Because of that, our further research broadened not only the portfolio of genes tested but also the variety of clinical diagnoses. Our hypothesis was that polycystic kidneys are the frequent manifestation accompanying number of nephropathies causing difficulties in clear differential diagnosis. This is even more complicated in families with intrafamilial alterations in phenotype, in young patients or prenatal cases with undeveloped phenotype or in families with no history of the disease or without the clinical data available. Moreover, combinations of mutations could cause untypical phenotype in patient.

Hence, our objectives included collection of groups of patients with wide range of renal phenotypes with corresponding clinical data and then genetic analysis of a panel of genes and genotype-phenotype correlation. Our panels were developed in collaboration with cooperating nephrologists, pediatricians and geneticists. For collection of clinical data, basic questionnaire for possible ciliopathic phenotypes was prepared and published. Regarding the laboratory part of the project, suitable method of sequencing was chosen and in-house bioinformatic pipeline developed.

The project was funded by grant projects of Charles University GAUK 1015 ('Molekulárně genetická diagnostika autozomálně recesivní polycystické choroby ledvin

metodou cíleného sekvenování nové generace') and PROGRES Q25/LF1. As this is the first project mapping the genetic background of Czech patients with polycystic kidney disease and at that time, the routine diagnosis of patients with polycystic kidneys was unavailable, the aim of this project was also to map the most frequent culprits of PKD in Czech patients. Moreover, genes responsible for noncystic types of nephropathies were included into the sequencing panel. This allowed rapid and cost-effective analysis benefiting from analysis of all patients with rare nephropathies running at once, together with testing of interest of collaborating physicians in diagnoses of specific noncystic nephropathies. Acquired knowledge regarding indicated disorders and effectivity of the sequencing method was later implemented into routine diagnostics. All results were reported back to the attending physician which, in some cases, allowed better patient and family counseling and eventually prenatal diagnosis.

SUBJECTS AND METHODS

Patients

The group of probands within our projects counted 149 patients and their 176 relatives. These patients were gathered at the Institute of Biology and Medical Genetics between years 2012 and 2019. The study was approved by the Ethics Committee of General University Hospital in Prague and all patients or legal guardians gave written informed consent for genetic testing. The basic characteristics of the whole cohort are summarized in Table 3.

Age at diagnosis	Cases	%
All	**149**	
Prenatal	26	17
Perinatal	5	3
Neonatal	22	15
Infantile	12	8
Childhood	20	14
Adolescence	3	2
Adulthood	57	38
Not disclosed	4	3
Sex	**Cases**	**%**
All	**149**	
Female	62	42
Male	69	46
Not disclosed	18	12

Table 3: The basic characteristics of the cohort.
Prenatal – before birth, Perinatal – from twenty-second week of gestation to 7 days after the delivery, Neonatal – newborn period until 28 days after the birth, Infantile – 29 days to 1 year, Childhood – period from 2 years until 13 years of age, Adolescence – 14 to 19 years of age, Adulthood – period from adolescence onward

In the first years (2012 to 2015), patients with clinically suspected ARPKD were collected and analyzed within the project of next-generation sequencing of the *PKHD1* gene. The clinical diagnosis was provided by attending geneticists or pediatricians.

After 2015, with introduction of panel and *PKD1* sequencing to our diagnostic algorithm, patients with wider clinical manifestation were included in our analyzed group of patients. Again, clinical diagnosis was provided by attending geneticists, pediatricians or nephrologists. There were no exclusion criteria for patients regarding the molecular

genetic analysis, except for patients who (or whose legal guardians) did not give informed consent for use of their DNA for research purposes or insufficient quality or concentration of input material (DNA, whole blood, etc.). The aim of our research was to map the most common nephropathies (with emphasis on cystic diseases) in the Czech Republic and to correlate the genetic results with the phenotype manifestation of the disease, hence even clinically unresolved cases were included in our group.

The group of patients was for the purposes of the research divided into 2 groups regarding their kidney phenotype to:

1. Cystic kidney diseases (i.e. ARPKD, ADPKD, RCAD syndrome and other non/systematic diseases with presence of renal cysts)
2. Kidney diseases without presence of cysts (i.e. focal segmental glomerulosclerosis, atypical hemolytic uremic syndrome, etc.)

Group 1 (cystic kidney diseases) comprised 128 probands and 170 relatives of the probands. The main analyzes (NGS) were provided for 134 patients, as in 4 probands, DNA sample was of low quality and/or concentration (samples from the termination of pregnancy) and DNA of proband's parents was analyzed instead; in one case trio of proband and his parents was analyzed. In the rest of the relatives, only Sanger resequencing of the detected variants was provided.
The layout of clinical diagnoses suspected in patients from Group 1 are summarized in Table 4.

Clinical diagnosis	Cases	%
All	**128**	
ARPKD	44	34
ADPKD	41	32
BBS/Meckel syndrome	4	3
NPHP	2	2
RCAD syndrome	3	2
OFD1	1	1
PKDTS	1	1
PKD of unknown etiology	32	25

Table 4: Clinical diagnoses in Group 1.
ARPKD – autosomal recessive polycystic kidney disease, ADPKD – autosomal dominant polycystic kidney disease, BBS – Bardet-Biedl syndrome, NPHP – nephronophthisis, RCAD syndrome – renal cysts and diabetes syndrome, OFD1 – orofaciodigital syndrome I, PKDTS – polycystic kidney disease, infantile severe, with tuberous sclerosis, PKD – polycystic kidney disease

Detailed clinical data were collected for patients from Group 1 and included: age at diagnosis, parental renal ultrasound, renal and hepatic phenotype of proband and extra-renal/hepatic manifestation (Supplementary Table S1). However, the clinical data of typical cases of ADPKD patients (usually with familial history of ADPKD) were not collected. Also, some clinical data are missing as they were not disclosed by attending clinicians.

Group 2 (noncystic nephropathies) comprised 21 analyzed probands and 6 relatives in whom only Sanger resequencing of detected variants was done. The clinical diagnoses provided for patients from group 2 are summarized in Table 5. No clinical data were collected for this group of patients as the analysis in these patients was used to verify the effectiveness of the molecular genetic analysis in patients with noncystic nephropathies. Moreover, we tested which types of nephropathies would be the most indicated by our collaborating geneticists/nephrologists for the purposes of introduction of molecular genetic analysis of noncystic nephropathies in the routine genetic testing.

Clinical diagnosis	Cases	%
All	**21**	
FSGS	6	28.5
aHUS	6	28.5
Gitelman syndrome	3	14
BOR1	1	5
Nail-patella syndrome	1	5
C3 nephropathy	1	5
CHRI	3	14

Table 5: Clinical diagnoses in group 2.
FSGS – focal segmental glomerulosclerosis, aHUS – atypical hemolytic uremic syndrome, BOR1 – branchiootorenal syndrome 1, CHRI – chronic renal insufficiency

DNA Isolation

Whole blood samples

Genomic DNA was isolated from whole blood samples by two purification methods depending on downstream applications.

Isolation with QIAamp DNA Mini Kit (Qiagene) was used for DNA purification of samples that were considered for sequencing on GS Junior instrument (more in chapter Next-generation sequencing) and/or samples meant for Sanger sequencing - especially samples of parents or other relatives of probands. In these patients only targeted (Sanger) resequencing for DNA variants already identified in probands was performed. Isolation was carried out on QIAcube (Qiagene) – instrument for automated DNA purification from 200 μL of whole blood sample. The isolation was carried out following the standard protocol recommended by Qiagene.

DNA isolation in samples of probands intended for capture-based sequencing was performed with Gentra Puregene Blood Kit (Qiagene) from 300 μL of whole blood by standard protocol.

Samples of amniotic fluid

QIAamp DNA Mini Kit (Qiagene) was used for DNA purification of samples of amniotic fluids. The isolation slightly differed depending on the input material:
Amniotic fluid: 2 – 3 mL of amniotic fluid was at first centrifuged 10 minutes at 3,000 rpm in 10°C, the supernatant was carefully discarded and 200 μL of PBS (phosphate-buffered saline) was added to the cells. The isolation was then carried out following the standard protocol recommended by QIAamp DNA Mini Kit (Qiagene) protocol on automatic isolator QIAcube (Qiagene).
Cultivated amniotic-fluid cells: 3 mL of PBS was added to the cultivated amniotic-fluid cells and the solution was centrifuged 10 minutes at 3,000 rpm in 10°C, the supernatant was carefully discarded and 200 μL of PBS was added to the cells. The isolation then continued following the standard protocol recommended by QIAamp DNA Mini Kit (Qiagene) protocol on automatic isolator QIAcube (Qiagene).

Next-generation sequencing

Next-generation sequencing (NGS) – also called parallel, high-throughput or deep sequencing was used for mutation analysis of all proband samples (in 4 cases of proband's parents due to low quality/concentration of the proband DNA). Two different sequencing technologies (and two types of sequencers) were used throughout the project – 454 sequencing (pyrosequencing) on the GS Junior (Roche Life Science) sequencer, and sequencing by synthesis (SBS) technology by Illumina performed on MiSeq sequencer (Illumina).

In the first years of project the sequencing on GS Junior sequencer was performed due to several reasons: (1) The project started with sequencing of only one gene – *PKHD1*, and the technology is ideal for this purpose (2) The availability of a GS Junior sequencer in the laboratory of Institute of Biology and Medical Genetics (3) Ideal platform for sequencing of smaller number of patients. In October 2013, Roche company announced they would stop supporting the platform by 2016. In 2015, we started with panel sequencing on MiSeq sequencer thanks to (1) Funding by The Charles University Grant Agency and Progres Program (2) Availability of MiSeq sequencer at the Institute of Pathology of the First Faculty of Medicine and General Teaching Hospital and thereafter also at the Institute of Endocrinology in Prague and General University Hospital in Prague. The SBS technology is ideal for sequencing of gene panels of several patients at once.

Both techniques with corresponding protocols are thoroughly described in manuals so I will only depict main characteristics of techniques mentioned, their usage in our project, and remark differences in our protocol, as the description of the whole wet lab processes would be lengthy and unnecessary.

454 sequencing

The process of sequencing is divided in number of steps and in general consists of library preparation of several samples, cleaning and multiplexing of samples, and sequencing itself.

Library was prepared from DNA obtained by isolation with QIAamp DNA Mini Kit (Qiagene). The fragments for sequencing were prepared in two steps: The first round of

PCR was prepared using target-specific primers with universal-tailed (M13) overhang (Generi Biotech) previously described in literature (Losekoot et al., 2005). The second round of PCR was prepared using primers containing universal tail (matching M13 adapter from the first round of PCR), specific MID (multiplex identifier) - sequences unique for the patient, and sequencing adaptors (see Figure 16)

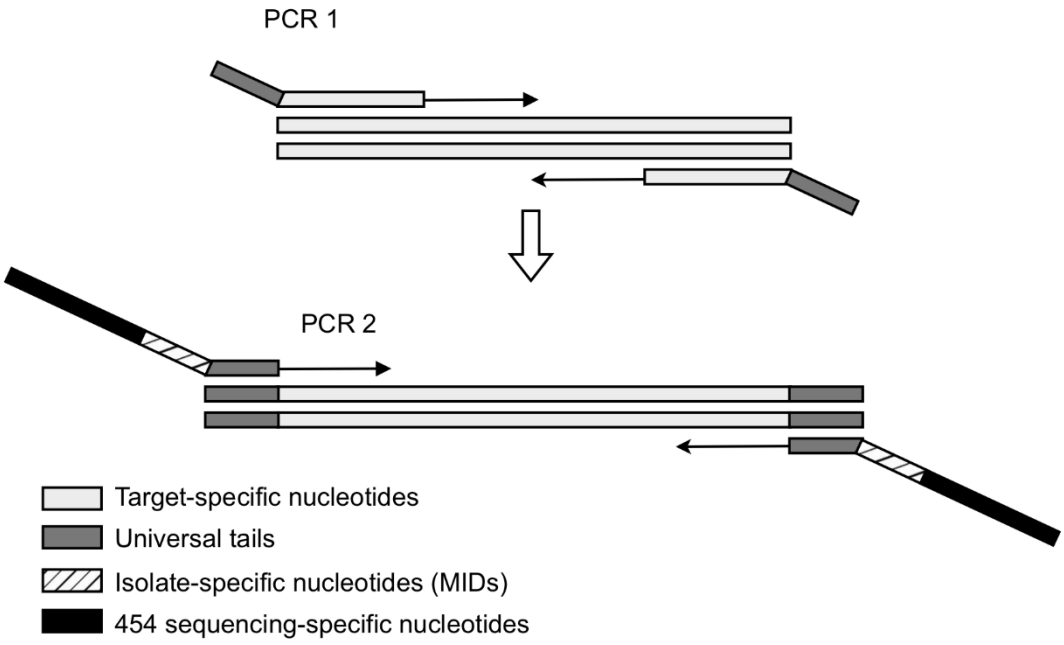


Figure 16: Design of two-round PCR strategy with fusion primers containing target-specific sequences and universal tail (PCR 1), and second PCR (PCR 2) with primers with universal tails, MID sequences and sequencing adaptors (consisting of key sequence, and A and B sequencing adaptors. Adapted from Boers et al., 2012.

The library of 87 amplicons (covering coding exons of the *PKHD1* gene, NM_138694.4) was then purified by Agentcourt® AMPure® XP (Beckman Coulter), the library of 6 patients was quantified (Quant-iT™ PicoGreen® dsDNA Assay Kit, Invitrogen), multiplexed equimolarly and prepared for sequencing run on GS Junior with kits GS Junior Titanium emPCR Kit (Lib-A) and GS Junior Titanium Sequencing Kit (Roche Diagnostics) in accordance to standard protocol.

The main steps of library preparation included:

1. Preparation of the Reagents and library for emulsification
   - Total number of 6 samples (patients) were prepared in one run of sequencing. This number of samples have proved to be optimal for required sequencing coverage
2. Emulsification
3. Amplification
4. Bead recovery and Cleanup
5. Library Bead Enrichment
6. Annealing of sequencing primers

The total amount of enriched beads prepared for the sequencing was then evaluated by GS Junior Bead Counter v2 (Roche Diagnostics). The ideal input of 500,000 enriched beads is recommended.
The library was loaded on the previously prepared PicoTiterPlate device together with beads containing reagents necessary for the sequencing.

Bioinformatics

Bioinformatic analysis of the sequencing data was done by commercial bioinformatic tool Sequence Pilot (JSI Medical Systems) – an automated platform for analysis of sequencing data that offers all-in-one tool SeqNext for mapping, alignment and variant detection. The sequencing data created by GS Junior sequencer – SFF files (Standard flowgram format) – were then uploaded into the Sequence Pilot and automatically analyzed with all parameters left in the original setting.

Data analysis and sequence changes classification

The sequenced samples were compared to the reference sequence of the *PKHD1* gene NM_138694.4 with 10 bp overhangs to the intron regions. Detected variants were checked with databases, such as Mutation Database Autosomal Recessive Polycystic Kidney Disease (http://www.humgen.rwth-aachen.de) and/or Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php). In case of novel mutations, their pathogenic potential was assessed. Nonsense or frameshift variants leading to a

premature STOP codon as well as exon deletion were considered as definitely pathogenic. The pathogenic potential of new missense variants was evaluated computationally using PolyPhen-2 (Adzhubei et al., 2010) and MutationTaster (Schwarz et al., 2014). Other two programs, NetGene2 (Brunak et al., 1991; Hebsgaard et al., 1996) and Human Splicing Finder (Desmet et al., 2009) were used to asses possible splice effect of intronic variants. Common polymorphisms were checked with Aachen PKHD1 database (see above) and/or NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/).

Sequencing by synthesis technology

SBS technology was used for sequencing of a panel of genes (sequencing with target enrichment) and the *PKD1* gene, respectively (sequencing using Nextera XT technology for library preparation). In both methods, the last step is sequencing on MiSeq sequencer, but the library preparation strategy is different.

Panel sequencing

For panel sequencing, we used DNA samples prepared by Gentra Puregene Blood Kit (Qiagene) purification. Due to the collaboration with nephrology and genetics departments from the whole Czech Republic, we sometimes obtained DNA already purified by another laboratory. As we had no information about elution buffer in these samples, we firstly changed the buffer of all samples. This must be done because of the first step of library preparation – fragmentation. As we use enzyme fragmentation, we must be sure there is no EDTA present in the buffer or we must know the concentration of EDTA in elution buffer. Since we had no information regarding the buffer, we purified DNA samples by ethanol precipitation with following protocol:

1. Add 1/10 volume of Sodium Acetate (3 M) and 2X volume (calculated after addition of sodium acetate) of chilled 95% ethanol
2. Incubate overnight at low temperature (-20ºC)
3. Centrifuge at 13,000 rpm for 20 minutes at - 4ºC
4. Discard supernatant carefully
5. Rinse with chilled 75% ethanol
6. Centrifuge at 13,000 rpm for 5 minutes at - 4ºC

7. Discard supernatant and dry the pellet at thermoblock heated to 40ºC ~ 15 minutes or until dry
8. Dissolve pellet in desired volume of 10 mM Tris-HCl

(Prior the ethanol-based precipitation, we also tried column-based DNA precipitation using Amicon™ low-binding Microcon YM-50 (MilliporeSigma™). Eventually, we decided to use ethanol-based precipitation as we achieved higher DNA yields and the method was cheaper as well.)

After the precipitation, the DNA samples were quantified using Qubit™ 2.0 Fluorometer (Invitrogen™) with Qubit™ dsDNA HS Assay Kit (Invitrogen™) and prepared for fragmentation. Gradually, the amount of input DNA was set to 500 ng (in initial experiments we started with lower amount of DNA, but higher input concentration proved better as lower number of replicates were present at the end of the sequencing protocol).

The subsequent steps were executed using Roche Sequencing Solutions: KAPA HyperPlus Kit (KR1145). This kit already contains fragmentation enzyme which is very sensitive on temperature and expiration date. For that reason, we later switched to separated packs of fragmentation enzyme - KAPA Frag Kit for Enzymatic Fragmentation (KR1141) in combination with KAPA Hyper Prep Kit (KR0961) for library preparation. The library preparation was carried out following the standard protocol using previously mentioned kits with Agentcourt® AMPure® XP (Beckman Coulter), and included:

7. Enzymatic Fragmentation
    - Time of fragmentation was optimized in accordance with previous results
    - Three samples were prepared for quality control on Agilent 2100 Bioanalyzer
    - Total number of 16 samples (patients) were prepared in one run of sequencing (we have chosen this number of samples based on our previous experience taking into account the optimal sequencing coverage, number of replicates and off-target reads)
8. End Repair and A-tailing
9. Adapter Ligation
    - Adapters used in our protocol were adapters by Roche Sequencing Solutions contained in SeqCap® Adapter Kit A and SeqCap® Adapter Kit B

10. Post-ligation Cleanup
11. Double-Sized Size Selection
    - Three samples were prepared for quality control on Agilent 2100 Bioanalyzer
12. Library amplification
    - With 500 ng of input DNA we used 4 cycles of amplification
13. Post-amplification Cleanup

All samples were then quantified using Qubit™ dsDNA HS Assay Kit (Invitrogen™) and 11 samples (3 samples after fragmentation step, 3 samples after size selection step and 4 samples after post-amplification cleanup) were analyzed on Agilent 2100 Bioanalyzer using Agilent High Sensitivity DNA Kit (Figure 17).

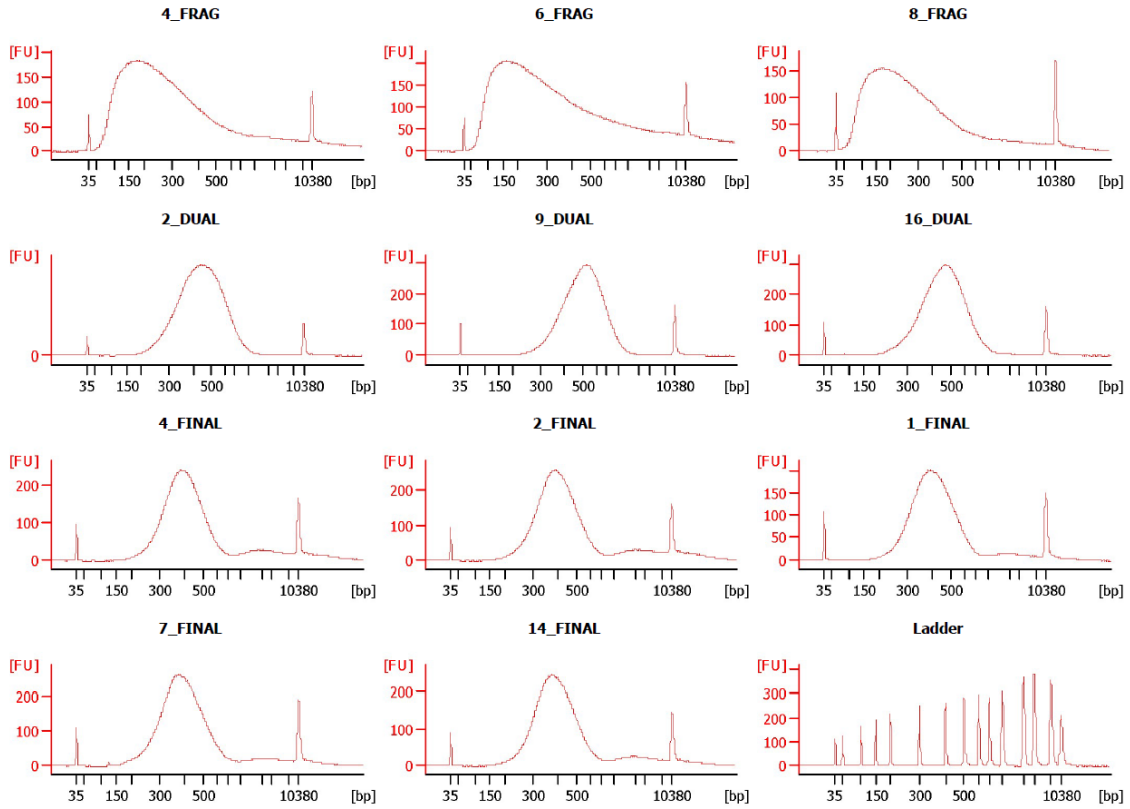


Figure 17: Example of the report from Agilent 2100 Bioanalyzer generated during one of our analysis. Samples after fragmentation: 4_FRAG, 6_FRAG, 8_FRAG, samples after double-sized size selection: 2_DUAL, 9_DUAL, 16_DUAL, samples after post-amplification cleanup: 4_FINAL, 2_FINAL, 1_FINAL, 7_FINAL and 14_FINAL.

After the quality control of samples, the library preparation continues following the protocol SeqCap® EZ Library SR User's Guide using kits by Roche Sequencing Solutions: SeqCap® Hybridization and Wash Kit, SeqCap® Accessory Kit V2, SeqCap®

HE-Oligo Kit A and SeqCap® HE-Oligo Kit B, SeqCap Pure Capture Bead Kit, and Agentcourt® AMPure® XP (Beckman Coulter). The SeqCap® EZ probe pool contained probes designed for our panel of genes (later in more detail).

The protocol included:

14. Preparation of multiplexed DNA Sample Library Pool
15. Preparation of Hybridization Sample
16. Hybridization
    - The recommended overnight hybridization was in our case changed to 2.5 day (from Friday afternoon to Monday morning) as the on-target yield was better with longer hybridization time.
17. Preparation of Capture Beads and binding of DNA to the Capture Beads
18. Washing of the Capture Beads Plus Bead-Bound DNA
19. Amplification of Captured Multiplex DNA Sample
20. Post-amplification Cleanup
21. Quality control (Agilent 2100 Bioanalyzer) and quantification (Qubit™ 2.0 Fluorometer)

The library was then prepared for sequencing on MiSeq sequencer following Illumina protocol MiSeq System, Denature and Dilute Libraries Guide (Document#15039740v10):

22. Denaturation and dilution of sequencing library
    - The concentration of library was always adjusted in regard to the previous results, nevertheless lately we used 14 pM as it seemed the cluster concentration was ideal for MiSeq Reagent Kit v3 (150-cycle).
23. Sequencing
    - In first years, we used MiSeq Reagent Kit v2 (300-cycle), lately we switched to MiSeq Reagent Kit v3 (150-cycle).

The SeqCap® EZ probe pool

The probes were designed with The NimbleDesign® - free online tool for custom probe selection for selected target. Our first panel (used between years 2015 and 2017) 'NefroPanel_1' contained 118 genes (Supplementary Table S3), the later design (used since 2018) called 'NefroPanel_2' contained 153 genes and covered about 0.5 Mbp of genomic DNA (Supplementary Table S4).

Sequencing of the *PKD1* gene

Because of the presence of 6 pseudogenes with high homology to *PKD1*, library preparation from long-range PCR products was chosen. The long-range products were prepared in 9 reactions (and included non-duplicated region as well, for easier analysis of all products at once). Primers and protocols used for the amplification were already described in (Tan et al., 2012). At first, GeneAmp® High Fidelity PCR System (Applied Biosystems) was used for long-range PCR. Later, the experiments with other polymerases, such as Q5® High-Fidelity DNA Polymerase (New England Biolabs) and PrimeSTAR® GXL DNA Polymerase (TaKaRa) was executed and polymerase by TaKaRa was chosen for its robustness and clean results of subsequent sequencing. The conditions of reactions were left as described before. The products were then checked on agar electrophoresis and multiplexed equimolarly (based on their length, concentration and previous results). After this step the library preparation was provided by Institute of Endocrinology in Prague with Nextera XT DNA Library preparation kit (Illumina). Samples were processed in group with other products during the shared runs. Sequencing was executed on MiSeq Sequencer using MiSeq Reagent Kit v2 (500-cycles).

Bioinformatics

Analysis of sequencing data from MiSeq sequencer has been done by in-house developed bioinformatic pipeline. The input data were compressed FASTQ files created by built-in software MiSeq Reporter in MiSeq sequencer. MiSeq Reporter provides secondary analysis of the base calls and quality scores generated during the sequencing run and creates FASTQ file that includes information about the read, sequence of a read and quality of all bases in the read. The FASTQ files are separately generated for each patient thanks to the sample sheet – a comma-separated values (CSV) file provided by a user

during the sequencing setup – containing information about list of samples and their index sequences that are essential for demultiplexing of the reads and their assignment to each sample. The final output of the bioinformatic analysis are VCF files (Variant Call Format) – a text files of annotated and filtered variants found in every patient. The next paragraphs will detail the bioinformatic analysis procedure we took in our group of patients and will briefly describe basic parameters and file formats used during the analysis.

The workflow developed and used for our analysis contains variety of open-source (publicly available) analysis tools of third parties running on Linux operating system.

1. Examination of raw reads quality

First, the quality of raw reads was evaluated. The Babraham Institute providing generally used tool FastQC also provides example reports of a good and a bad sequencing run (see http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The mapping statistics generated by FastQC tool are summarized in HTML report and compressed text files for every sample analyzed.

Tool used:	FastQC v0.11.5
Input:	SAMPLE_R1.fastq.gz
	SAMPLE_R2.fastq.gz
Output:	SAMPLE_R1_fastq.html
	SAMPLE_R1_fastq.zip
	SAMPLE_R2_fastq.html
	SAMPLE_R2_fastq.zip

```
/path/to/fastqc *.fastq.gz
```

2. Adapter trimming and quality filtering

The next step includes cutting off the adapter sequences found in the reads and also checking quality of each read and their trimming. The tool provides packages with different types of adapter sequences generally used in sequencing protocols. In our

project we used TruSeq3-PE-2.fa package of adapters. Moreover, in case of *PKD1* sequencing, detection and deletion of possible PCR primer sequences was added.

Tool used: Trimmomatic 0.36 (Bolger et al., 2014)
http://www.usadellab.org/cms/?page=trimmomatic

Input: SAMPLE_R1.fastq.gz
SAMPLE_R2.fastq.gz

Output: SAMPLE_trimmed_1P.fastq (paired)
SAMPLE_trimmed_2P.fastq (paired)
SAMPLE_trimmed_1U.fastq (unpaired)
SAMPLE_trimmed_2U.fastq (unpaired)

```
java -jar /path/to/trimmomatic-0.36.jar PE \
SAMPLE_R1.fastq.gz SAMPLE_R2.fastq.gz \
SAMPLE_trimmed_1P.fastq SAMPLE_trimmed_1U.fastq \
SAMPLE_trimmed_2P.fastq SAMPLE_trimmed_2U.fastq \
ILLUMINACLIP/path/to/adapters/TruSeq3-PE-2.fa:2:30:10 \
TRAILING:20 \
SLIDINGWINDOW:5:20 \
MINLEN:50 \
&> trimmomatic.log
```

3. Quality check of trimmed reads

In this step we checked the quality of trimmed paired reads that were used for mapping to reference genome.

Tool used: FastQC v0.11.5

Input: SAMPLE_trimmed_1P.fastq
SAMPLE_trimmed_2P.fastq

Output: SAMPLE_trimmed_1P_fastqc.html
SAMPLE_trimmed_1P_fastqc.zip
SAMPLE_trimmed_2P_fastqc.html
SAMPLE_trimmed_2P_fastqc.zip

```
/path/to/fastqc *P.fastq
```

4. Mapping to reference genome

After the quality check and adjustment of raw reads the reads were mapped to reference genome. In our project the bwa.kit tool was used. Bwa.kit is a tool containing packages of scripts providing read mapping, generation of human reference genome hs38DH, and is capable of performing HLA typing etc. The hs38DH genome provided by bwa.kit consists of GRCh38 primary assembly plus additional ALT contigs, decoy sequences and HLA genes. More information regarding hs38DH and bwa.kit, respectively, can be found at:
https://github.com/lh3/bwa/blob/master/README-alt.md
https://github.com/lh3/bwa/tree/master/bwakit.

Tool used: bwa.kit 0.7.12
Input: SAMPLE_trimmed_1P.fastq
SAMPLE_trimmed_2P.fastq
Output: SAMPLE.aln.bam

```
/path/to/bwa.kit/run-bwamem \
-o SAMPLE.aln.bam \
-R "@RG\tID:"$SAMPLE_NAME"\tSM:"SAMPLE_NAME"" \
/path/to/hs38DH/hs38DH.fa \
SAMPLE_trimmed_1P.fastq SAMPLE_trimmed_2P.fastq | sh
```

5. Clean up and Sorting

This step consists of cleaning up of pair read information (fixmate) and sorting of aligned reads by their leftmost coordinate (sort).

Tool used: samtools 1.6
http://www.htslib.org/doc/samtools.html
Input: SAMPLE.aln.bam
Output: SAMPLE_fixed_sorted.bam

```
/path/to/samtools fixmate -O bam SAMPLE.aln.bam
SAMPLE_fixed.bam

/path/to/samtools sort SAMPLE_fixed.bam >
SAMPLE_fixed_sorted.bam
```

6. Marking and removal of duplicates

To avoid bias in variant calling, the PCR duplicates were marked and deleted in this step.

Tool used: picard MarkDuplicates 2.9.2-SNAPSHOT
https://broadinstitute.github.io/picard/command-line-overview.html#MarkDuplicates

Input: SAMPLE_fixed_sorted.bam

Output: SAMPLE_fixed_sorted_dedup.bam

```
java -jar /path/to/picard.jar MarkDuplicates \
I=SAMPLE_fixed_sorted.bam \
O=SAMPLE_fixed_sorted_dedup.bam \
M=SAMPLE_MarkDuplicates.txt \
REMOVE_DUPLICATES=true \
ASSUME_SORTED=true \
CREATE_INDEX=false
```

7. Indexing

The sorted BAM files were then indexed (to allow fast access) and index file was created.

Tool used: samtools index 1.6
http://www.htslib.org/doc/samtools.html

Input: SAMPLE_fixed_sorted.bam

Output: SAMPLE_fixed_sorted_dedup.bam.bai

```
/path/to/samtools index SAMPLE_fixed_sorted_dedup.bam
```

8. Collecting of basic run metrics

Statistics regarding sequencing run were evaluated in several steps following below. The basic run metrics done within our panel sequencing project are summarized in Supplementary Table S5.

Alignment evaluation:

Tool used: picard CollectAlignmentSummaryMetrics 2.9.2-SNAPSHOT

http://broadinstitute.github.io/picard/command-line-overview.html#CollectAlignmentSummaryMetrics

Input: SAMPLE_sorted.bam

Output: SAMPLE_AlignMetrics.txt

```
java -jar /path/to/picard.jar CollectAlignmentSummaryMetrics
I=SAMPLE_sorted.bam \
O=SAMPLE_AlignMetrics.txt \
R=/path/to/hs38DH/hs38DH.fa
```

Calculation of insert size of reads:

Tool used: picard CollectInsertSizeMetrics 2.9.2-SNAPSHOT

http://broadinstitute.github.io/picard/command-line-overview.html#CollectInsertSizeMetrics

Input: SAMPLE_sorted_dedup.bam

Output: SAMPLE_InsertSize.txt

```
java -jar /path/to/picard.jar CollectInsertSizeMetrics
I=SAMPLE_sorted_dedup.bam \
O=SAMPLE_InsertSize.txt \
H=$bname.pdf
```

Calculation of percentage of on/off target reads:

Tool used: picard CollectHsMetrics 2.9.2-SNAPSHOT

http://broadinstitute.github.io/picard/command-line-overview.html#CollectHsMetrics

Input: SAMPLE_sorted_dedup.bam

Output: SAMPLE_hs_metrics.txt

```
java -jar /path/to/picard.jar CollectHsMetrics \
I=SAMPLE_sorted_dedup.bam \
O=SAMPLE_hs_metrics.txt \
R=/path/to/hs38DH/hs38DH.fa \
BAIT_INTERVALS=/path/to/panel_primary_targets.interval_list
TARGET_INTERVALS=/path/to/panel_primary_targets.interval_lis
t
```

Calculation of depth of coverage:

Depth of coverage calculation and visualization was done using slightly changed scripts by Stephen Turner:

https://www.gettinggeneticsdone.com/2014/03/visualize-coverage-exome-targeted-ngs-bedtools.html

First, bedtools coverage was used to calculate coverage in all bases included in the panel. Then, results were visualized by script in R programming language (RStudio was used for visualization, https://www.rstudio.com/). Example of 3 samples are showed in Figure 18.

Tool used: bedtools coverage v2.26.0
https://bedtools.readthedocs.io/en/latest/content/tools/coverage.html

Input: ALL_SAMPLES_fixed_sorted_dedup.bam

Output: ALL_SAMPLES.hist.all.txt

```
/path/to/bedtools coverage \
-hist -b ALL_SAMPLES_fixed_sorted_dedup.bam \
-a /path/to/panel_primary_targets.bed | grep ^all > ALL_SAMPLES.hist.all.txt
```

Code in R:

```
print(files <- list.files(pattern="all.txt$"))
print(labs <- paste("samp", gsub("prefixToTrash-
0|\\.pe\\.on\\.pos\\.dedup\\.realigned\\.recalibrated\\.bam\
\.cov\\.hist\\.txt\\.all\\.txt", "", files, perl=TRUE),
sep=""))
cov <- list()
cov_cumul <- list()
for (i in 1:length(files)) {
  cov[[i]] <- read.table(files[i])
  cov_cumul[[i]] <- 1-cumsum(cov[[i]][,5])
}
library(RColorBrewer)
cols <- brewer.pal(length(cov), "Dark2")

png("exome-coverage-plots.png", h=1000, w=1000,
pointsize=20)

plot(cov[[1]][2:401, 2], cov_cumul[[1]][1:400], type='n',
xlab="Depth", ylab="Fraction of capture target bases \u2265
depth", ylim=c(0,1.0), main="Target Region Coverage")
abline(v = 20, col = "gray60")
abline(v = 50, col = "gray60")
abline(v = 80, col = "gray60")
abline(v = 100, col = "gray60")
abline(h = 0.50, col = "gray60")
abline(h = 0.90, col = "gray60")
axis(1, at=c(20,50,80), labels=c(20,50,80))
axis(2, at=c(0.90), labels=c(0.90))
axis(2, at=c(0.50), labels=c(0.50))

for (i in 1:length(cov)) points(cov[[i]][2:401, 2],
cov_cumul[[i]][1:400], type='l', lwd=3, col=cols[i])

legend("topright", legend=labs, col=cols, lty=1, lwd=4)

dev.off()
```

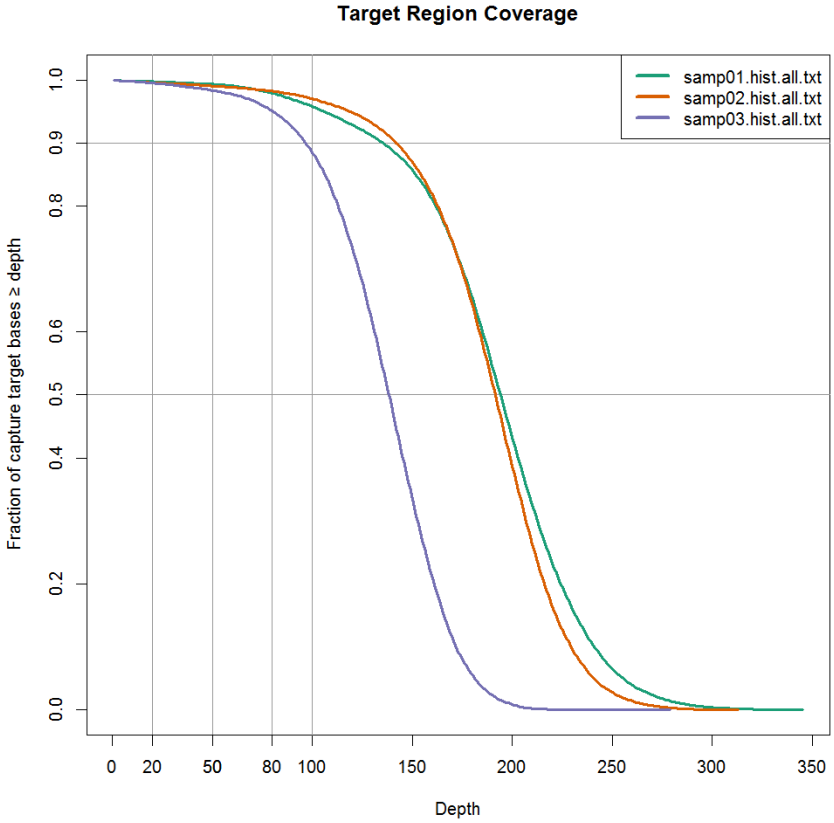


Figure 18: Visualization of depth of coverage in 3 samples.

Compilation of all log files into single report

Tool used: MultiQC v1.1

https://multiqc.info/

Input: All available log files created by other programs

Output: Summary statistics on all analyzed samples

Summary html report

```
/path/to/multiqc .
```

9. Variant calling

The variants were called using Bayesian genetic variant caller Freebayes. The caller is designed to find single-nucleotide polymorphisms (SNPs), small insertions and deletions, multi-nucleotide polymorphisms (MNPs) and smaller complex events. The regions of variant calling were restricted by BED file to only comprise genes (coding exons and exon-intron boundaries) included in our panel. This step already incorporates hard

filtering of low-quality variants (or artefacts of sequencing) that filters out reads with low quality, variants with low number of observations, variants with observations only on one (reverse or forward) type of reads or variants that originates only on certain part of reads. The minimal coverage of the site to call the variant was in our case set to 8 reads. The fraction of observations supporting alternate allele was set to the default of 0.2.

Tool used: freebayes v1.1.0-9-g09d4ecf (Garrison and Marth, 2012)
https://github.com/ekg/freebayes

Input: SAMPLE_fixed_sorted_dedup.bam

Output: SAMPLE_freebayes.vcf.gz

```
/path/to/freebayes \
--fasta-reference /path/to/hs38DH/hs38DH.fa \
--bam SAMPLE_fixed_sorted_dedup.bam \
--targets /path/to/panel_primary_targets.bed \
--genotype-qualities \
--no-partial-observations \
--min-repeat-entropy 1 \
--min-coverage 8 \
--min-mapping-quality 10 \
--min-base-quality 10 \
| vcffilter --info-filter "QUAL > 1 & QUAL / AO > 10 & SAF >
0 & SAR > 0 & RPR > 1 & RPL > 1" \
| vcfallelicprimitives --tag-parsed DECOMPOSED --keep-geno \
| vcfbreakmulti \
| vcffixup - \
| vcfstreamsort \
| vt normalize -n -r /path/to/hs38DH/hs38DH.fa -q >/dev/null
| vcfuniqalleles \
| bgzip -c > SAMPLE_freebayes.vcf.gz
```

10. Decompression of VCF files

Tool used: gunzip 1.6
https://en.wikibooks.org/wiki/Guide_to_Unix/Commands/File_Compression#gunzip

Input: SAMPLE_freebayes.vcf.gz

Output: SAMPLE_freebayes.vcf

```
Path/to/gunzip -c SAMPLE_freebayes.vcf.gz >
SAMPLE_freebayes.vcf
```

11. Variant annotation

The variants found were then annotated and their functional effect on genes and proteins was predicted.

Tool used: SnpEff 4.3r (Cingolani et al., 2012a)
http://snpeff.sourceforge.net/SnpEff.html
Input: SAMPLE_freebayes.vcf
Output: SAMPLE_freebayes_SnpEff.vcf

```
java -jar /path/to/snpEff.jar eff \
-canon \
-hgvs \
-noLog \
-i vcf \
-o vcf \
-stats SAMPLE-effects-stats.html \
GRCh38.86 \
SAMPLE_freebayes.vcf > SAMPLE_freebayes_SnpEff.vcf
```

The variants were subsequently annotated using population databases. In our case:
dbSNP (https://www.ncbi.nlm.nih.gov/snp/)
dbNSFP 3.2a (https://sites.google.com/site/jpopgen/dbNSFP) compiling information regarding prediction scores from prediction programs, conservation scores, allele frequencies from large sequencing projects, etc.

Tool used: SnpSift 4.3r (Cingolani et al., 2012b)
Input: SAMPLE_freebayes_SnpEff.vcf
Output: SAMPLE_freebayes_SnpEff_SnpSift.vcf

```
java -jar /path/to/SnpSift.jar annotate \
-dbsnp -db /path/to/dbSNP/GRCh38/00-All.vcf.gz
-v SAMPLE_freebayes_SnpEff.vcf \
| java -jar /path/to/SnpSift.jar dbnsfp \
-db /path/to/dbNSFP/GRCh38/dbNSFP3.2a.txt.gz -v - >
SAMPLE_freebayes_SnpEff_SnpSift.vcf
```

The variants and their corresponding annotations were then extracted to .txt file.

Tool used: SnpSift 4.3r (Cingolani et al., 2012b)

Input: SAMPLE_freebayes_SnpEff.vcf

Output: SAMPLE_freebayes_SnpEff_SnpSift_filtered.txt

```
java -jar /path/to/SnpSift.jar extractFields \
-s "," \
-e "." \
SAMPLE_freebayes_SnpEff_SnpSift.vcf \
"CHROM" "POS" "ID" "REF" "ALT" "QUAL" "GEN[*].GT"
"ANN[*].HGVS_C" "ANN[*].HGVS_P" "DP" "RO" "QR" "AO" "QA"
"SRP" "SAP" "TYPE" "MQM" "MQMR" "DECOMPOSED" "AF"
"ANN[*].EFFECT" "ANN[*].IMPACT" "ANN[*].GENE"
"ANN[*].GENEID" "ANN[*].RANK" "ANN[*].CDS_POS"
"ANN[*].AA_POS" "dbNSFP_Interpro_domain" "G5" "G5A" "CAF"
"COMMON" "TOPMED" "dbNSFP_1000Gp3_AF" "dbNSFP_ExAC_Adj_AF"
"dbNSFP_1000Gp3_EUR_AF" "dbNSFP_ExAC_NFE_AF"
"dbNSFP_ESP6500_EA_AF" "dbNSFP_MetaSVM_pred"
"dbNSFP_Polyphen2_HDIV_pred" "dbNSFP_Polyphen2_HVAR_pred"
"dbNSFP_SIFT_pred" "dbNSFP_LRT_pred"
"dbNSFP_MutationAssessor_pred" "dbNSFP_MutationTaster_pred"
"dbNSFP_PROVEAN_pred" "dbNSFP_FATHMM_pred"
"dbNSFP_CADD_phred" "dbNSFP_phastCons100way_vertebrate"
"dbNSFP_GERP___RS" "dbNSFP_GERP___NR" "PM" "PMC"
"LOF[*].GENE" "NMD[*].GENE" "ANN[*].ERRORS" \
> SAMPLE_freebayes_SnpEff_SnpSift.txt
```

12. Filtration

Hard filtration of the low-quality or unlikely variants was already incorporated in the variant calling step (step 9). In this step, filtration of variants based on their probable causality was done. In each sample about 400-600 variants have been usually found. These variants must have been therefore filtered to narrow the group of assessed variants. We filtered the variants based on these parameters:

- The variant causes changes on protein level (i.e. sequence changes in exons and without synonymous changes) or
- The variant is in the intronic region in the donor (+1, +2, +4, +5) or acceptor (-1, -2) splite site.

- The variant has a population frequency (of European descend) lower than 0.5. Usually 1000 Genome Project EUR population was chosen, sometimes Exome Aggregation Consortium (ExAC) was added in filtration. The filtration parameter was later toughened up to 0.1.
- The INFO field of dbSNP database "G5" is set as a FALSE. The G5 field indicates the variant has a >5% minor allele frequency in one or more populations (https://www.ncbi.nlm.nih.gov/variation/docs/oldglossary_dbSNP1_vcf/).

The possible causality of remaining variants was then assessed considering *in silico* predictions in programs: SIFT (Sorting Intolerant From Tolerant) (Sim et al., 2012), PolyPhen-2 (Polymorphism Phenotyping v2**)** (Adzhubei et al., 2010), LRT (Likelihood Ratio Test) (Chun and Fay, 2009), MutationTaster (http://www.mutationtaster.org/), MutationAssessor (Reva et al., 2011), FATHMM (Functional Analysis through Hidden Markov Models) (Shihab et al., 2013), PROVEAN (Protein Variation Effect Analyzer) (Choi et al., 2012), CADD (Combined Annotation Dependent Depletion) (Rentzsch et al., 2019), ensemble scores MetaSVM (meta-analytic support vector machine) that combines values from multiple deleteriousness prediction methods (Dong et al., 2015), and conservation scores.
The variants were also consulted with VarSome search engine (Kopanos et al., 2019) containing data retrieved from archive of clinically relevant interpretations of variant ClinVar (Landrum et al., 2016), protein database Uniprot (Bateman et al., 2017) etc. and reporting variant pathogenicity using automated classifier evaluating variants according to the ACMG guidelines (Richards et al., 2015).

Suspected variants were evaluated considering all mentioned criteria together with ACMG scores computed by VarSome and with consideration to clinically suspected disease and its inheritance. The consultation with attending nephrologist or geneticist was often made to discuss detected variants without known pathogenicity. In 2017, the questionnaire of clinical data for analyzed patients was released at the web pages of General University Hospital in Prague ('Dotazník k mol. genet. vyšetření u nemocných se suspekcí na autozomálně recesivní polycystickou chorobu ledvin (ARPKD) a dalších cystických onemocnění ledvin (ciliopatií)',

https://www.vfn.cz/wp-content/uploads/2019/07/f-ublg-51-018-dotaznik-arpkd-ciliopatie.pdf). The form covered wide range of manifestations of ciliopathic syndromes and was intended to ease the genotype-phenotype correlation in patients. Nonetheless, the attending physicians often sent the patient without (correctly) filled clinical data which made the evaluation of variants difficult. Because of that, we are planning to be strict in these requirements and analyze only patients with filled all necessary forms.
The final genetic report to attending physician included written evaluation of the genetic results and possible recommendation of further genetic analysis of patients and/or his/her relatives.

13. Copy number calling

The aligned sequencing reads (BAM files) from all samples in the same run were also used for calling of large genome rearrangements. A copy-number reference compilated from pool of samples was used to evaluate each sample. The results were then visualized. For detailed process of copy-number variant calling see https://cnvkit.readthedocs.io/en/stable/pipeline.html

Tool used: CNVkit 0.8.6.dev0 (Talevich et al., 2016)
Input: ALL_SAMPLES_fixed_sorted_dedup.bam
Output: SAMPLE_freebayes_SnpEff_SnpSift_filtered.txt

```
# Genome preparation

/path/to/cnvkit.py access /path/to/hs38DH.fa \
-o access.ds38DH.bed
```

```
# Target preparation

/path/to/cnvkit.py target path/to/panel_capture_targets.bed
--annotate path/to/refFlat.txt \
--split \
--avg-size 100 \
-o panel_capture_targets_cnv_annotated_avg_100.bed
```

```
# Antitarget preparation

/path/to/cnvkit.py antitarget
path/to/panel_capture_targets_cnv_annotated_avg_100.bed \
-g access.ds38DH.bed \
-o panel_antitargets.bed
```

```
# Coverage Calculation in target and antitarget bins

path/to/cnvkit.py coverage SAMPLE_fixed_sorted_dedup.bam
panel_capture_targets_cnv_annotated_avg_100.bed \
-o SAMPLE.targetcoverage.cnn

path/to/cnvkit.py coverage SAMPLE_fixed_sorted_dedup.bam
panel_antitargets.bed \
-o SAMPLE.antitargetcoverage.cnn
```

```
# Reference preparation

/path/to/cnvkit.py reference *.targetcoverage.cnn \
-f /path/to/hs38DH.fa \
-o REFERENCE.cnn
```

```
# Copy-number variant calling and visualization

/path/to/cnvkit.py batch \
ALL_SAMPLES_fixed_sorted_dedup.bam
-r REFERENCE.cnn \
--scatter \
--diagram
```

Sanger sequencing

All probable causal mutations found by NGS were always confirmed in patients and (if available) their parents/relatives by Sanger sequencing done on capillary electrophoresis instrument ABI PRISM 3130 (Life Technologies). The confirmation of variant positions in relatives of proband is extremely important especially in cases of autosomal recessive inheritance to verify the variants are not located on the same chromosome.
Sanger sequencing was also used for resequencing of the *PKD1* gene regions with low coverage (under 20x) in the next-generation sequencing of the *PKD1* by Nextera XT kit.

MLPA

The MLPA (Multiplex ligation-dependent probe amplification) analysis of *PKHD1* and *HNF1B* genes was usually done in patients without 2 causal mutations found by *PKHD1* sequencing or patients with suspected RCAD syndrome or prenatal cases. The kits used

for MLPA analysis were SALSA MLPA P341 PKHD1 mix 1 and P342-PKHD1 mix 2 (MRC Holland) and SALSA MLPA P241 MODY Mix 1. MLPA was also used for confirmation of large deletions found by panel sequencing, for example SALSA MLPA P387 NPHP1 for whole gene deletion of the *NPHP1* gene. The software used for the analysis of MLPA data was Coffalyser.Net (MRC Holland).

RESULTS

The group of probands analyzed within our project counted 149 patients and their 176 relatives. For the purposes of the research, patients were divided into two groups regarding their kidney phenotype: Group 1 (cystic nephropathies) and Group 2 (noncystic nephropathies).

Group 1 (cystic nephropathies)

Group 1 counted 128 probands and 170 relatives. The types of clinical diagnoses with corresponding number of patients are summarized in Table 4. The main analyzes were provided for 134 patients (in 4 probands DNA was of low quality/concentration and samples of the parents were used instead for the NGS analyses, in one case, trio was used). In the rest of the relatives, Sanger resequencing of the detected variants was provided. The different types of analyses provided for each proband can be found in Supplementary Table S6. The list of all probands from Group 1 with their collected clinical data, clinical diagnoses and detected sequence variants with definitive genetic diagnoses is in Supplementary Table S1.

ARPKD was clinically diagnosed in 44 patients from the Group 1. The molecular genetic analysis confirmed the clinical diagnosis of ARPKD (2 probably causal variants in the *PKHD1* gene in trans) in 23 patients (52%) (Table 6). In 5 patients (11%), 2 probably causal variants in the *TMEM67* gene were detected and genetic diagnosis of nephronophthisis 11 was made (results of one patient were reported from commercial laboratory – patient n. 52). Three patients (7%) harbored mutation in the *PKD1* gene and final genetic diagnosis was ADPKD.

In 13 patients (30%) the genetic analysis did not detect probable causal variant/s and genetic diagnosis remained unknown. Nevertheless, in 2 of these patients another diagnosis was later reported from the attending clinician (patient n. 19 – bilateral blastema nephroblastoma and possible Perlman syndrome; patient n. 24 – Mayer-Rokitansky-Kuster-Hauser syndrome). Also, in 2 patients suspected variant in another gene with unknown effect on final phenotype was found (patient n. 25 with variant in *NPHP4* gene; patient n. 34 with variant in the *COL4A3* gene). Interestingly, in 7 patients (16%) only one *PKHD1* mutation was found.

Genetic diagnosis	Cases	%
All	**44**	
ARPKD	23	52
NPHP	5	11
ADPKD	3	7
Unknown	13	30

Table 6: Genetic diagnoses in patients with suspected ARPKD.

In 41 patients, clinical diagnosis of ADPKD was made. In 68% (28 patients) the clinical diagnosis was confirmed by molecular genetic analysis and final diagnosis of ADPKD was made (Table 7). In 13 patients (32%) the molecular genetic analysis did not detect probable causal mutation in the patient. Unsurprisingly, the mutational detection yield was higher in patients with positive family history of ADPKD. From 28 patients with familial history of ADPKD, the genetic analysis detected causal mutation in 23 patients (82%). In five patients with positive family history (18%) no causal variant was identified. On the other hand, in 8 patients without family history of ADPKD, causal mutation in the *PKD1* gene was detected in 2 patients (25%), in 6 patients (75%) no variant was detected.

Genetic diagnosis	Cases	%
All	**41**	
ADPKD	28	68
Familial history of ADPKD	23	82
No history of ADPKD in family	2	7
Not disclosed	3	11
Unknown	13	32
Familial history of ADPKD	5	38.5
No history of ADPKD in family	6	46
Not disclosed	2	15.5

Table 7: Genetic diagnoses in patients with suspected ADPKD and their family history of ADPKD.

In the less common diagnoses with phenotype that usually includes extrarenal and extrahepatic manifestation, the molecular genetic diagnosis usually corresponded to the predicted clinical diagnosis. Specifically, in 4 patients with suspected Bardet-Biedl or Meckel syndrome the diagnosis was confirmed in three of them. In one patient (patient n.

124) no probable causal variant was identified, nevertheless suspected finding from array CGH analysis was later reported.
Clinical diagnosis of NPHP was confirmed in one of two suspected patients. In the latter, suspected likely pathogenic variant in the *WT1* gene was detected (patient n. 61).
From three patients with suspected RCAD syndrome, the clinical diagnosis was confirmed in 2 patients. In the third patient suspected variant in the *RET* gene was found (patient n. 85).
The diagnoses of orofaciodigital syndrome and syndrome of polycystic kidney disease with tuberous sclerosis were confirmed by molecular analysis in both cases.

In 32 patients, the clinical diagnosis was unclear.
In 12 of these patients, the suspicion of attending clinician was ARPKD or another cystic phenotype, such as ADPKD, RCAD syndrome etc. The genetic findings confirmed ARPKD in 4 patients (33%), RCAD syndrome in 2 patients (17%) and NPHP11 in one patient (8%) (Table 8). In 5 patients (42%) the genetic diagnosis remained unknown.

In 19 patients, the clinical diagnosis was unspecified with only phenotype of cystic kidneys/cystic kidney dysplasia indicated. In those patients, molecular genetic analysis detected ARPKD in one patient (5%), suspicion of another diagnosis in 7 patients (37%) and the genetic diagnosis remained unknown in 11 patients (58%).
Seven patients with detected suspected variant in other genes were: patients n. 83 and 84 – siblings with *PAX2* partial deletion detected; patient n. 78 with variant in *PAX2*; patient n. 81 – with suspected variant in the *SIX2* gene; patient n. 126 – sample from termination of pregnancy with deletion in the *TRIM32* gene and missense variant in the *MKS1* gene identified; patient n. 63 with combination of missense variant in *PKHD1* and nonsense variant in *TMEM237* and patient n. 68 with mosaic missense variant in the *PKD1* gene detected in 20% of reads. More details of the patients and their genetic findings are in Discussion.

In one patient with suspected RCAD syndrome or NPHP (patient n. 128), whole gene deletion of *HNF1B* was identified and RCAD syndrome confirmed.

Genetic diagnosis	Cases	%
All	**32**	
Suspected ARPKD or another cystic nephropathy	12	38
ARPKD	4	33
RCAD syndrome	2	17
NPHP11	1	8
Unknown	5	42
PKD of unknown etiology	19	59
ARPKD	1	5
Suspicion of different diagnosis	7	37
Unknown	11	58
RCAD syndrome or NPHP	1	3

Table 8: Genetic diagnoses in patients with phenotype of cystic kidneys/cystic kidney dysplasia without definitive clinical diagnosis.

Group 2 (noncystic nephropathies)

Group 2 counted 21 probands and 6 relatives and comprised patients with noncystic nephropathies. The detailed clinical data was not collected for these diagnoses as the inclusion of these diagnoses to the sequencing panel served more likely to map demands of clinicians for molecular genetic diagnosis for purposes of routine diagnosis. Summarized information including patients, their clinical diagnoses and detected variants are in Supplementary Table S2.

The most indicated diagnoses were focal segmental glomerulosclerosis (FSGS) and atypical hemolytic uremic syndrome (aHUS) – both with 6 patients (Table 9). Genetic analysis confirmed FSGS in one patient (17%), 6 patients did not have causal mutation identified (83%). aHUS was confirmed in 2 patients (33%), in remaining 4 patients genetic analysis did not detect causal variant (67%). However, in one patient with clinically diagnosed aHUS (patient 18b), variant in the *PAX2* gene was detected by panel sequencing.

In 3 patients, Gitelman syndrome was clinically diagnosed. In 2 patients (2 brothers, 5b and 6b) diagnosis was confirmed by panel sequencing. One patient remained without detected causal variant.

Three patients were sent to the molecular analysis with general phenotype of chronic renal insufficiency. In one patient (8b) possible causal variant in the *INF2* gene was detected and genetic diagnosis of FSGS made. One patient (21b) harbored missense

variant with uncertain significance in the *UMOD* gene causing phenotype of medullary cystic kidney disease 2/familial juvenile hyperuricemic nephropathy (MDCK2/FJHN). In both of these cases, attending clinician agreed the clinical phenotype of patient could correspond to the phenotype caused by mutations in these genes. One patient remained genetically unresolved.

Patients with clinically suspected branchiootorenal syndrome and nail-patella syndrome were confirmed on genetic level. In patient with suspected C3 nephropathy, no variant was detected.

Genetic diagnosis	Cases	%
All	**21**	
FSGS	6	28.5
FSGS	1	17
Unknown	5	83
aHUS	6	28.5
aHUS	2	33
Suspicion of different diagnosis	1	17
Unknown	3	50
Gitelman syndrome	3	14
Gitelman syndrome	2	67
Unknown	1	33
Chronic renal insufficiency	3	14
FSGS	1	33.3
MDCK2/FJHN	1	33.3
Unknown	1	33.3
Branchiootorenal syndrome 1	1	5
Nail-patella syndrome	1	5
C3 nephropathy	1	5

Table 9: Genetic diagnoses in patients with noncystic nephropathies.
FSGS – focal segmental glomerulosclerosis; aHUS – atypical hemolytic uremic syndrome; MDCK2/FJHN – medullary cystic kidney disease 2/familial juvenile hyperuricemic nephropathy.

Overall, from all 149 patients, the analyses carried out within our project yielded final genetic diagnosis in 84 patients (56%). In remaining 65 patients (44%), the genetic analysis did not detect causal variant/variants or raised the suspicion on another diagnosis. Also, in 6 samples, the clinical diagnosis was later changed by attending physician or the results of molecular genetic analysis were reported from another laboratory. Regarding the division into two groups, the yield of final genetic diagnosis

was slightly higher in group of cystic kidney diseases (59%) versus noncystic kidney diseases (43%).

The lower yield of detected causal variants was noted in prenatal cases. From 24 prenatal cases analyzed within our project, only 6 samples (25%) showed agreement in clinical and genetic diagnosis, in 4 samples (17%) the genetic analysis detected another causal variant and 14 cases (58%) remained genetically unsolved. The lower percentage of findings and high number of misdiagnoses in prenatal cases may be caused by still undeveloped phenotype with non-specific finding of renal hyperechogenicity on the prenatal ultrasound. This makes the discrimination of the disease etiology very difficult or even impossible (Tsatsaris et al., 2002).

On the contrary, the prenatal/perinatal cases with perinatal death were usually correctly clinically diagnosed, as all 3 cases with suspected ARPKD harbored 2 causal mutations in the *PKHD1* gene (Figure 19). This finding corresponds with the fact that typical severe form of ARPKD consist of prenatally enlarged kidneys with oligohydramnios and pulmonary hypoplasia causing respiratory distress (Guay-Woodford et al., 2014), which was found in all of these patients. Surprisingly, one case of prenatal manifestation of the disease with perinatal death remained unsolved, although typical findings indicating severe ARPKD were described in the patient (patient n. 61). Nevertheless, suspicion on NPHP was stated by attending clinician and not all clinical data were disclosed to the laboratory, so we do not have full clinical picture of the patient.

Overall, in samples from terminated pregnancies, the mutational detection achieved the diagnosis in 31% (5 patients). Higher yield – 75% (3 patients) – was reached in prenatally diagnosed cases with perinatal death. Patients with prenatally diagnosed PKD who survived perinatal period reached the diagnosis in 62.5% (5 patients). In children and adolescents with postnatally made diagnosis, the mutational detection was successful in 66% (41 patients) and quite similar yield was achieved in adult patients – with 63% (24 patients). The yields of mutational detection and types of diagnoses within different age groups of patients are summarized in Figure 19. Our results are slightly lower compared to study by (Bullich et al., 2018), where mutational detection reached the diagnosis in 79% of terminated pregnancies, 86% of prenatally diagnosed patients who survived the perinatal period, 72% in postnatally diagnosed children and 80% of adults.

However, the comparison is difficult as our inclusion criteria were quite open and even patients with unspecific clinical findings were included.

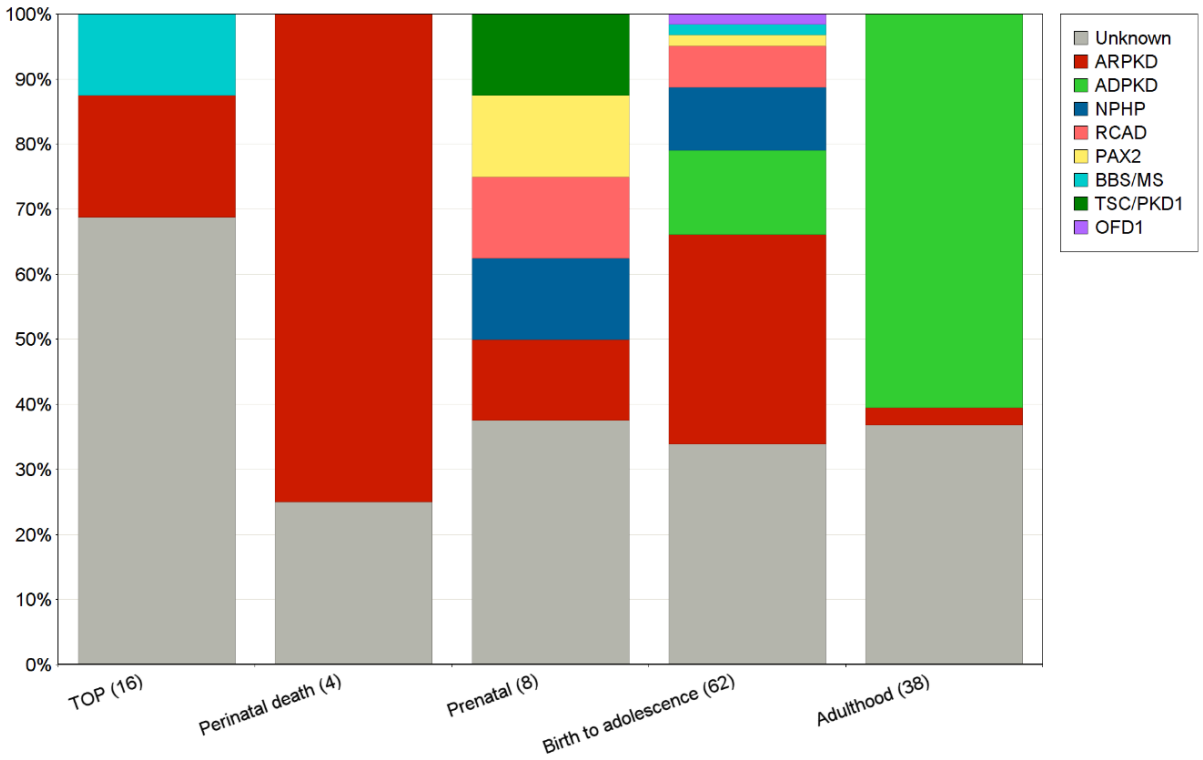


Figure 19: Disease distribution according to disease onset.
ARPKD – autosomal recessive polycystic kidney disease, ADPKD – autosomal dominant polycystic kidney disease, NPHP – nephronophthisis, RCAD – renal cysts and diabetes syndrome, PAX2 – Papillorenal syndrome, BBS/MS – Bardet-Biedl and Meckel syndrome, OFD1 – orofaciodigital syndrome, TSC2/PKD1 - TSC2/PKD1 contiguous gene syndrome.

DISCUSSION

The analyses carried out within our project yielded final genetic diagnosis in 84 patients (56%) from group of 149 patients. In 65 patients (44%), the genetic diagnosis remained unknown or suspicion on other disease was raised.

The emphasis of our project lies in molecular genetic analysis of ARPKD. In the first years, the genetic diagnosis comprised only analysis of patients with suspected ARPKD as in that time no other laboratory provided molecular genetic analysis of the *PKHD1* gene. Over the time of this project 44 probands with clinically diagnosed ARPKD was sent to our laboratory. In 23 of them (52%) the diagnosis was confirmed and two probably causal mutations of the *PKHD1* gene were detected in trans. The important clinical sign of ARPKD are, besides typical renal phenotype, abnormalities in liver (Guay-Woodford, 2015). This fact mirrors our results regarding yield of genetic diagnosis in clinically diagnosed ARPKD patients. From 21 patients with clinically suspected ARPKD but unknown genetic diagnosis, liver abnormalities were presented only in 33% (7 patients). Thirteen patients (62%) had normal liver phenotype, in one patient the information was not disclosed. On the contrary, from 28 patients with genetically confirmed ARPKD (regardless clinical diagnosis), 19 of them (68%) had liver abnormality, 7 of them (25%) had normal liver findings and in 2 (7%) liver phenotype was not disclosed. The importance of corresponding liver phenotype was also described in our article where partial results were presented (Obeidova et al., 2015).

Interesting findings with an impact on our routine diagnosis was identification of five (11%) patients with NPHP11 in our clinically diagnosed ARPKD patients (in one patient – patient n. 52 – results from commercial laboratory were reported), and additional 2 patients with *TMEM67* mutations with other clinical diagnosis. Extensive study published in 2011 counting 440 patients with NPHP-related ciliopathies detected 26 patients (from 20 families) with recessive *TMEM67* mutations (Chaki et al., 2011). Liver fibrosis was presented in 16 of these families, abnormalities in CNS in 10, mental retardation in 14 and retinal coloboma in 8 of these families. Also, authors noted they never detected patient with 2 nonsense mutations – this is true for our patients as well. In our patients, liver fibrosis was detected in 3 patients, in 3 patients the hepatic findings were normal and in one patient the information was not disclosed. Eyes abnormalities manifested in our patients were oculomotor apraxia (patient n.1) and nystagmus (patient

n. 32). Oculomotor apraxia was also described in patient with NPHP11 in (König et al., 2017). Although results by Chaki et al. indicate the CNS abnormalities are very common in NPHP11, no one of our patients showed abnormal CNS phenotype. This fact may be caused by type of mutation detected in our patients. Five patients harbored homozygous mutation p.Cys615Arg, one patient harbored this mutation in combination with another *TMEM67* mutation. Mutation p.Cys615Pro was already described by Otto et al., 2009 as hypomorphic allele associated with phenotype of NPHP with hepatic fibrosis and no brain anomaly. That would correspond with our finding that the only patient without p.Cys605Arg was sample of fetus from termination of pregnancy (i.e. severe form of the disease). Moreover, in the study by Otto et al., haplotype analysis using highly polymorphic microsatellite markers revealed a shared haplotype indicating inheritance of the p.Cys615Arg mutation from a common ancestor.

In conclusion, due to relatively high number of patients with *TMEM67* variant in our group of patients, and especially in patients with clinically diagnosed ARPKD, we added *TMEM67* to our routine diagnosis of patients with ARPKD, as it seems the differential diagnosis of ARPKD and NPHP11 is complicated or NPHP11 is sometimes underestimated or overlooked in clinical practice.

Relatively high number of patients had only one probably pathogenic variant in the *PKHD1* detected. One *PKHD1* variant without any other variant detected was in 7 patients, additional 5 patients had combination of *PKHD1* variant and variant in another gene identified. These findings are in concordance with literature, as single heterozygous *PKHD1* variant was detected in 36% of 164 ARPKD patients in study by prof. Bergmann (Bergmann et al., 2005a).

The study of obligate carriers (parents of ARPKD patients) executed in 2011 showed that carrier status for ARPKD is a predisposition to renal and liver involvement including increased renal medullary echogenicity and asymptomatic polycystic liver disease with hepatic fibrosis (Gunay-Aygun et al., 2011). Nevertheless, phenotype of carriers described is much less severe than in our patients.

The findings of relatively high number of patients with single heterozygous variant in *PKHD1* can have several possible reasons.

First, the method of analysis can miss some mutation. Nevertheless, some of the patients were analyzed by 454 sequencing of the *PKHD1* gene and then by SBS panel sequencing. The results were always the same.

Second, the variant can be missed by our analysis as it is located in a deep intronic region, other regulatory region or even in one of the alternate exons of the *PKHD1*. *PKHD1* has several alternate exons (more in Onuchic et al., 2002). However, three studies in which analysis of alternate *PKHD1* exons was also done did not detect causal variant or described variants with unknown significance (reviewed in Gunay-Aygun et al., 2010). The causal variant in deep intronic variant was already described in one publication (Michel-Calemard et al., 2009). In this article, 4 families were described with variant IVS46+653A>G which causes insertion of the new out-of-frame pseudoexon responsible for formation of a premature stop codon in exon 47. We cannot rule out the presence of variant with possible effect on final phenotype of our patients in a region that is not analyzed.

High proportion of ARPKD cases negative for *PKHD1* mutation or with only one detected mutation were investigated in publication by Szabó et al., 2018. The results of this study showed that screening for *PKHD1* CNVs in patients with a heterozygous point mutation is recommend. However, in our group of patients with single *PKHD1* mutation, MLPA of *PKHD1* was done in 10 of 13 patients (in one case parents of proband were analyzed) and no gene rearrangements were identified.

Another possibility is that combination of mutation in the *PKHD1* and another gene could cause the final phenotype in patient. The combination of mutations causing severe type of PKD was described in several families with dominant *PKD1/PKD2* mutation and mutation in *PKHD1/HNF1B* (Bergmann et al., 2011). In our case the combinations were *PKHD1* variant and: *PKD1* variant in patient n. 10 (segregation showed that one variant was inherited from father, one from mother); *ACTN4* (FSGS) variant in patient n. 30 (DNA samples of parents were not available); *ZNF423* (NPHP14) in patient n. 39 (DNA samples of parents were not available); *NPHP3* deletion of several exons in patient n. 43, and *TMEM237* variant in patient n. 63 (segregation showed that one variant was inherited from father, one from mother, healthy sister had neither of the variants). Although in the first 3 patients the second variants are predicted to be likely benign, the *NPHP3* deletion and *TMEM237* variant are likely to be pathogenic.

Two patients within suspected ARPKD carried variant in another gene. It was patient n. 25 and patient n.34. Patient n. 25 was a boy with a neonatal manifestation of increasing number of renal cysts and renal pelvis dilatations with no extrarenal symptoms (liver phenotype has not been disclosed). The boy carried variant p.Arg961His in the *NPHP4* gene. Although autosomal recessive disorder, number of patients with single

heterozygous *NPHP4* mutations were described (Hoefele et al., 2005). In the publication by Hoefele et al., German patient with the same variant was described. He/she as well carried one p.Arg961His variant and also manifested phenotype of renal cysts without any extrarenal symptoms. The study noted that findings of single heterozygous *NPHP4* variants were more than 3 times more frequent than findings of two *NPHP4* mutations in their large group of patients. They considered several reasons for these findings: technical reasons causing missing of second variant, location of mutation in regulatory elements, dominant effect of the variants or digenic or oligogenic inheritance described already in Bardet-Biedl or Meckel syndrome (Katsanis et al., 2001; Leitch et al., 2008; Zaghloul et al., 2010).

Patient n. 34 with prenatally detected enlarged kidneys with polyhydramnios and number of postnatal symptoms was clinically diagnosed as ARPKD. However, already described likely pathogenic variant in the *COL4A3* gene was detected. *COL4A3* is associated with autosomal recessive but also dominant (Jefferson et al., 1997) inheritance of Alport syndrome and benign hematuria (Badenas et al., 2002). Alport syndrome is characterized by hematuria, renal failure, hearing loss and eye abnormalities (Savige et al., 2013), but the phenotype can vary from full clinical picture of Alport syndrome to benign types of hematuria. The different severity was even described in patients with heterozygous mutations in *COL4A3* – from asymptomatic carriers to patients with fully developed features of the disease (Heidet et al., 2001). Unfortunately, although attending clinician was made aware of these findings and asked to assess the possibility of this finding regarding the patient's phenotype, no answer has been obtained yet. The genetic diagnosis is therefore still considered as unknown.

Overall, 69 variants in the *PKHD1* gene were detected in our group of patients; with 13% (9) of nonsense variants, 20% (14) of frameshift variants, one intronic variant, one deletion of exon 62 and 64% (44) missense variants. This roughly corresponds to study of 164 ARPKD patients conducted by prof. Bergmann where missense variants accounted for about 77% of all variants and truncating variants (nonsense and frameshift) for 23% (Bergmann et al., 2005a).

Missense variant p.Thr36Met (T36M) was detected in 20% of all findings (14x). T36M is a well-known recurrent variant occurring in every *PKHD1* mutational study, and constituting (which precisely corresponds with our findings) approximately every fifth

mutated *PKHD1* allele (Bergmann et al., 2005b). Although missense variant, T36M is associated with rather severe phenotype as it possibly represents a potential alternative initiation codon that is predicted to be stronger than the native start codon (Furu et al., 2003). This also corresponds with our findings that T36M was, in combination with nonsense or frameshift variant, found in 4 of 6 prenatal cases with termination of pregnancy or perinatal death. Besides T36M, *PKHD1* has no other mutational hotspot and variants are located throughout the whole gene.

In clinically diagnosed patients with ADPKD, the mutational detection rate reached 68%. Unsurprisingly, the yield was higher in probands from families with history of ADPKD – 82%. The unclear outcome of sequencing analysis came out in patient n. 68. In this girl, mosaic missense variant in the *PKD1* gene was detected. The variant was identified with 20% of allele frequency which was confirmed also by Sanger resequencing. The cases of somatic mosaicism were already described in families with ADPKD, nevertheless the carriers of mosaic variant were either asymptomatic (even with nonsense mutation with high allele frequency in blood sample) (Connor et al., 2008) or mildly affected (Reiterová et al., 2013). Our patient had ADPKD with very early onset and missense *PKD1* variant of uncertain significance with 20% allele frequency from blood sample. Although, it is possible that allele frequency in patient's kidneys is higher, we could not make the final diagnosis based on this finding.

Overall, ADPKD was genetically proven in 31 patients from our analyzed set. Only mutations in the *PKD1* gene were detected. The majority of variants were frameshift (36.4%) variants, nonsense and missense variants accounted for 24,3% each. Intron variants were found in 3 cases (9%) – two of them were brothers, and also in two siblings, deletion of one amino acid was detected (6%). Partial deletion of *PKD1* together with partial deletion of *TSC2* was detected in patient n. 90 with correctly diagnosed TSC2/PKD1 contiguous gene syndrome. This corresponds to percentages found in study of 324 ADPKD patients, where missense mutations formed about 19%, truncating about 67% and in-frame deletion about 13% of all patients (Rossetti et al., 2002).

ADPKD is characterized by high allelic heterogeneity where no single mutation accounts for more than 2% of the total patient population (Bergmann et al., 2018). This was also observed in our group of patients. The presence of same mutation was observed only 3

times. Two times in siblings and one time in two, to our knowledge, unrelated patients (patient n. 91 and 99).

In group of patients with clinically diagnosed PKD of unknown etiology, seven patients harbored mutations that raised the suspicion on final diagnosis: patients n. 83 and 84 – siblings with *PAX2* partial deletion detected; patient n. 78 with variant in *PAX2*; patient n. 81 – with suspected variant in the *SIX2* gene; patient n. 126 – sample from termination of pregnancy with deletion in the *TRIM32* gene and missense variant in the *MKS1* gene identified; patient n. 63 with combination of missense variant in *PKHD1* and nonsense variant in *TMEM237* and patient n. 68 with mosaic missense variant in the *PKD1* gene detected in 20% of reads.

Patients 83 and 84 were brother and sister with healthy parents who had early manifestations of microcystic disease of unknown etiology. Moreover, both had eyes abnormalities. Panel sequencing detected deletion of several exons of *PAX2* gene in both siblings (NM_003990.5, exons 7 to 11). Hence, the suspicion on papillorenal syndrome was raised. Papillorenal syndrome (OMIM:120330) or renal-coloboma syndrome is a disorder caused by mutations in the *PAX2* gene with autosomal dominant inheritance. The typical manifestation of papillorenal syndrome includes bilateral optic nerve colobomas and renal hypoplasia. Additional symptoms can also include vesicoureteral reflux (VUR, condition in which urine flows backward from the bladder to one/both ureters or to the kidneys), auditory anomalies, CNS anomalies, and skin and joint anomalies (reviewed in Ecoles and Schimmenti, 1999). These symptoms would correspond to the disease manifestation in both children. The deletion is yet to be confirmed by another method and DNA of probands healthy parents will be as well analyzed. Nevertheless, we do not expect to find the deletion in the parents and rather suspect germline mosaicism in one of them (germline mosaicism has been already described in family with 3 affected siblings with *PAX2* mutation (Amiel et al., 2000)).

Interestingly, *PAX2* variant was detected in yet another patient. Patient n. 78 is a woman with unknown PKD phenotype indicated by attending clinician. Also, clinician noted her nephew has severe VUR. In this patient intron variant (c.497-2A>G, intron 4) in the *PAX2* gene was identified. Although this particular variant is described for the first time, intron variant IVS4-1G>T was already described in patient with papillorenal syndrome (Thomas et al., 2011). The variant is predicted to be pathogenic by automatic prediction

generated by VarSome (Kopanos et al., 2019) following ACMG criteria (Richards et al., 2015). Thus, we believe the variant could be causal in the patient.

Patient n. 81 was sent to the laboratory with unclear clinical diagnosis. He was an adult man with smaller kidneys with multiple cysts. No additional symptoms were noted by the clinician. The panel sequencing in this patient detected variant p.Pro241Leu in the *SIX2* gene. This variant was described two times in literature with different conclusions. In the first publication, the variant was described in several patients with renal hypodysplasia (Weber et al., 2008). Within the study functional studies were provided and the conclusion was that abnormalities in the *SIX2* gene are associated with defects of early kidney development. However, the study noted that pathogenesis of the disease is complex with multifactorial and/or polygenic actions and that this fact mirrors the incomplete penetrance in parental heterozygous mutation carriers. In the second publication, the variant was again detected in parental carriers and thus assessed as benign (Hwang et al., 2014).

Another unclear diagnosis was in patient n. 126. In this case, sample of the terminated fetus was sent to our laboratory, together with blood samples of both parents. Thus, trio was analyzed by panel sequencing. The results showed *TRIM32* (BBS11) deletion of great part of the gene inherited from father, with *MKS1* (Meckel syndrome 1) missense variant p.Arg164His inherited from mother. Mutations in *TRIM32* were identified in one family with Bardet-Biedl syndrome (Chiang et al., 2006). Recessive mutations in *MKS1* are associated with lethal phenotype of cystic dysplasia with occipital encephalocele, biliary dysgenesis and polydactyly (Consugar et al., 2007). The prenatal cases with Meckel syndrome were studied in (Chaumoitre et al., 2006) with findings comprising cystic kidneys, oligohydramnios (in 78% of cases) and additional manifestations of occipital defects and vermian agenesis. We can only speculate about effect of both variants on the final phenotype of the fetus, nevertheless combinations of *MKS1* and *BBS* (but not *TRIM32/BBS11*) variants in compound heterozygous or trialellic form (heterozygous in one gene and homozygous in second gene) were described in literature (Leitch et al., 2008). In this article combinations of missense *MKS1* variants with truncating (frameshift or nonsense) homozygous or heterozygous variants in the *BBS1*/*BBS10* genes were described. Unlike our patient, patients in the publication were children between ages 7-10 years with additional symptoms, such as seizures, deafness or dental anomalies. Unfortunately, to explore the possible effect of both variants/genes on

the disease development would require extensive functional studies which are not feasible in our laboratory.

The findings suggesting germline (or somatic) mosaicism in family were made in three cases during our project. Two brothers (patients n. 73 and 74) harbored same mutation in the *PKD1* gene while in neither of unaffected parents the variant was identified. In this case, paternity was done and showed that both parents are indeed the birth parents of the brothers. Another case was already mentioned – brother and sister (patients n. 83 and 84) with *PAX2* partial deletion. Although the DNA of parents was not yet analyzed, the deletion seems to be the culprit of the disease in their children, so we do not expect it to be present in heterozygous state in blood samples of the parents. Nevertheless, the paternity was not tested in this case. The last case is of proband n. 100. In this family pathogenic variant in *PKD1* was identified in proband and her maternal half-sister. However, their unaffected mother did not carry the mutation in blood or buccal sample. Hence, we suspect the variant is present in gametes and possibly other tissues of the mother.

In conclusion, the germline mosaicism (as well as somatic mosaicism or presence of hypomorphic alleles) must be considered in families without apparent family history of the autosomal dominant disease. In cases of ADPKD, number of families with described germline (or somatic) mosaicism is still increasing in the literature (Iliuta et al., 2017; Reiterová et al., 2013; Tan et al., 2015).

Regarding the findings in the group of patients with clinically diagnosed noncystic nephropathies the molecular genetic analysis yielded final genetic diagnosis in 43% (9 patients). In 11 cases (52%) the genetic diagnosis remained unknown. In one case (patient n. 18b) likely pathogenic variant in the *PAX2* gene was detected. The patient was sent to our laboratory with clinical diagnosis of aHUS. The final genetic diagnosis is here set as unknown as discussion with the attending clinician is still in process.

Another patient with unknown diagnosis is patient n. 2b. In this patient FSGS was diagnosed. However, panel sequencing discovered missense variant in the *TTC21B* gene. Although its mutations typically cause autosomal recessive NPHP11 (or phenotype of short-rib thoracic dysplasia 4 with or without polydactyly), cases of autosomal dominant inheritance were described (Davis et al., 2011). Also oligogenic combinations with

another ciliary genes were described (Davis et al., 2011). In this publication, patient with the same single heterozygous variant was described, however no clinical data were provided. As no functional study can be done in our laboratory, the genetic diagnosis is set to unknown.

An interesting case was patient n. 8b send to us with unclear diagnosis of chronic renal insufficiency. Panel sequencing detected missense variant in the *INF2* gene. This gene is associated with FSGS phenotype of unknown inheritance. However, in 2010 the publication emerged where heterozygous mutations in the *INF2* gene segregated with the disease in several families (Brown et al., 2010). Luckily, the same variant as ours was detected in large family with FSGS within their project. In this family, the mutation segregated with the disease, with age at diagnosis between 22 to 45 years and end-stage renal disease occurring in 4 from 10 affected individuals between 23 and 30 years of age.

Emerging demand from collaborating physicians is for genetic testing of atypical hemolytic uremic syndrome. Atypical HUS manifests with acute kidney injury, thrombocytopenia and microangiopathic hemolytic anemia, and at least 50% of patients have an underlying inherited and/or acquired complement abnormality (reviewed in Goodship et al., 2017). The genetics of aHUS is complicated by several factors. The final phenotype can be caused by mutations in several genes and also by combined heterozygous mutations in two of these genes (Rodríguez De Córdoba et al., 2014). Moreover, the polymorphisms acting as a risk or protective factors affecting the disease predisposition have been described (Caprioli et al., 2003; Noris et al., 2010). The value of segregation analysis in family is also decreased by only approximately 50% penetrance of aHUS-associated genes (Rodríguez De Córdoba et al., 2014). Our laboratory received 6 patients with clinically suspected aHUS. In two patients, aHUS was confirmed by molecular genetic analysis. However, in healthy relatives of one patient (patient n. 3b), the variant was also found. This may be caused by reduced penetrance of the allele or by benign effect of the allele on the phenotype of the patient.

We are aware of the limitations of our study. In the first place, it is the complicated algorithm of performed methods that differ in individual patients. This is caused by gradual implementation of various methods throughout the years and also by complications in *PKD1* gene analysis. However, new method is now tested in our

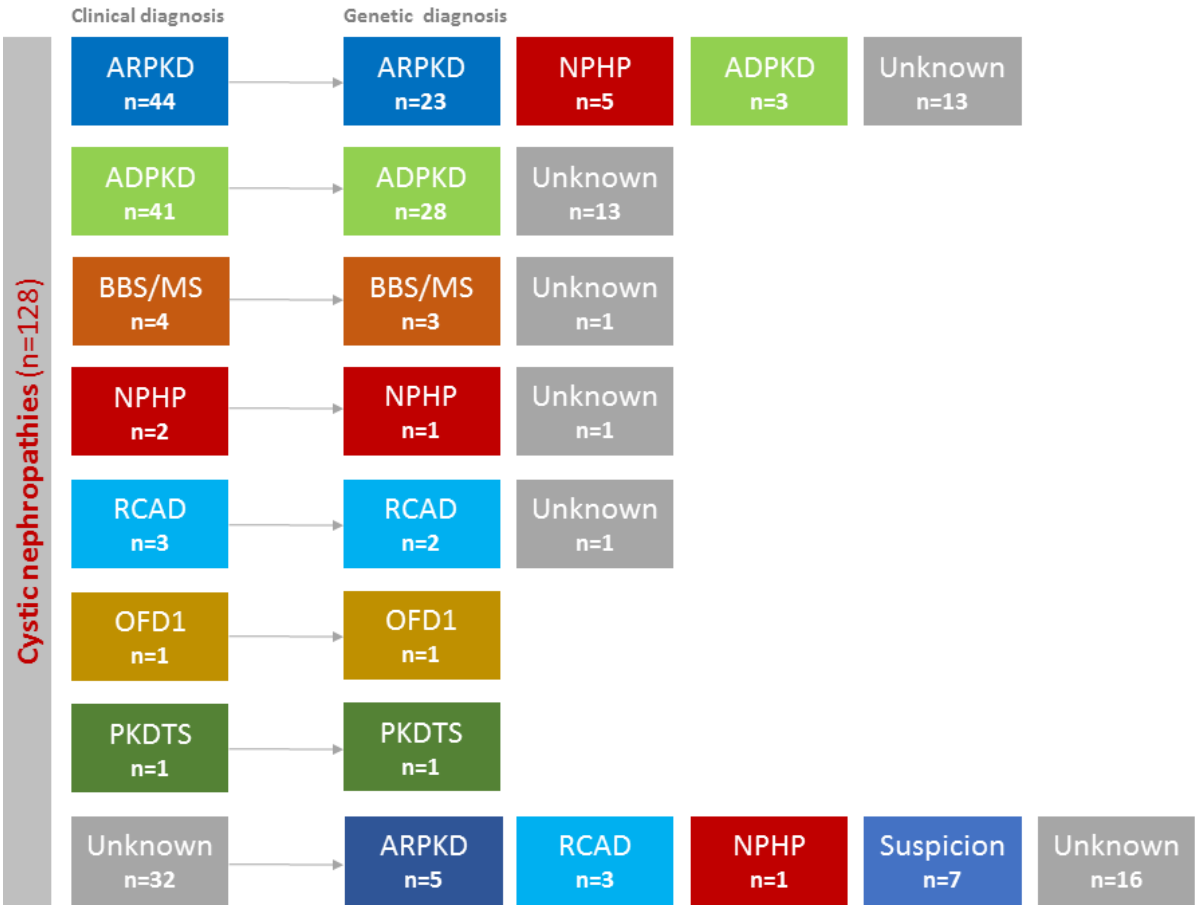
laboratory where *PKD1* can be sequenced within standard panel of genes. Also, with introduction of panel sequencing, CNV analysis can be provided by bioinformatic processing and MLPA analysis can be used just for confirmation.

Great emphasis is now on bioinformatic processing of sequencing data. The whole process is very complex with number of steps, and thus prone to mistakes. The final evaluation of variant pathogenicity is the central problem of the assessment of genetic diagnosis. Throughout the project the uncomplete clinical data or even unclear clinical diagnosis complicated evaluation of an indistinct variants. Also, segregation analysis was usually prolonged or impossible due to non-available DNA samples of proband's relatives. Because of that, DNA samples of parents will be required at least in the suspected disease with recessive inheritance and in cases of prenatal diagnosis. Also, questionnaire with clinical data will be required. Another step to variant evaluation is without question functional analysis. Nowadays, this is impossible in our laboratory as it is mostly adapted for routine genetic diagnostics.

CONCLUSION

The group of 149 patients with cystic and noncystic nephropathies and their 176 relatives was analyzed between years 2012 and 2019 at the Institute of Biology and Medical Genetics. The methods used for the sequencing analyses evolved from sequencing of the *PKHD1* gene in the beginning of the project to panel sequencing comprising at first 118 genes, later 153 genes.

The analyses carried out within our project yielded final genetic diagnosis in 84 patients (56%). In remaining 65 patients (44%), the genetic analysis did not detect causal variant/variants or raised the suspicion on another diagnosis. In group of patients with cystic diseases (128 patients) the mutational detection yield was slightly higher reaching 59% versus 43% in noncystic kidney diseases (21 patients) (Figure 20).



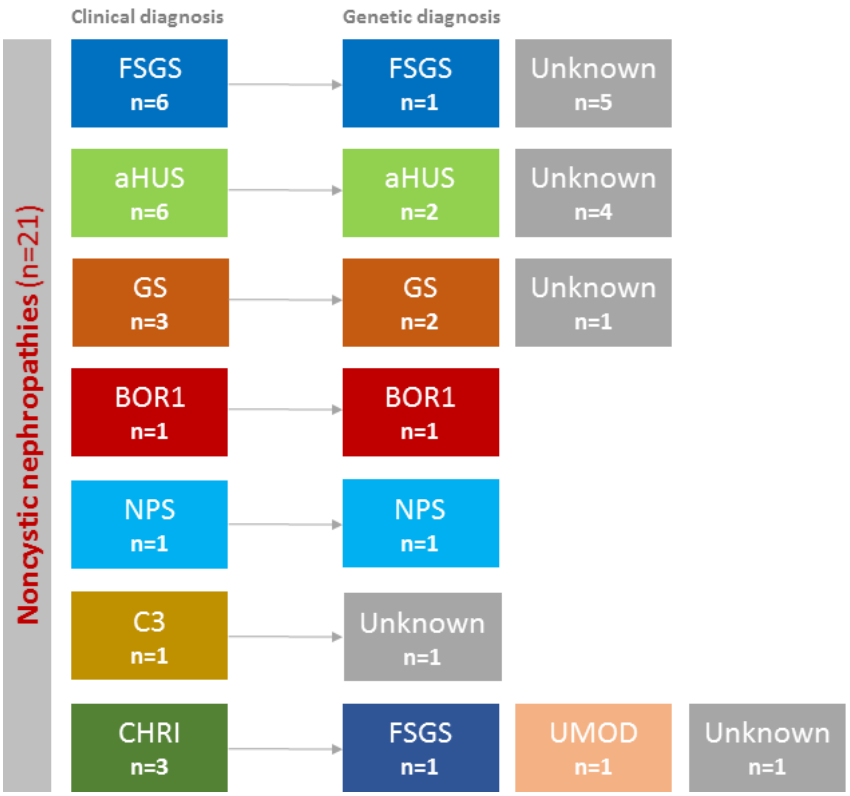


Figure 20: The clinical and genetic diagnoses in group of 128 and 21 patients with cystic and noncystic nephropathies, respectively.

To conclude, a panel of genes associated with formation of nephropathies allows comprehensive and rapid diagnosis of kidney diseases even in cases with ambiguous or not fully developed phenotype. The correct final diagnosis allows better care of the patient, as it can avoid unnecessary diagnostic procedures, sets the prognosis of the disease, and enables extrarenal comorbidities to be detected and treated early. Lastly, it enables genetic counselling for other family members including prenatal diagnostics for the next pregnancies of the parents.

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SUPPLEMENTARY

Supplementary Table S1: Group of patients with cystic nephropathies.

N.	Patient number
dbSNP	ID of variant in the NCBI database of genetic variations
ACMG	Automatic prediction of pathogenicity following criteria by American College of Medical Genetics
Inheritance	P – father, M – mother

Variants in italics are additional detected variants with unknown effect on patient's phenotype

ARPKD	Autosomal recessive polycystic kidney disease
ADPKD	Autosomal dominant polycystic kidney disease
BBS	Bardet-Biedl syndrome
NPHP	Nephronophthisis
OFD1	Orofaciodigital syndrome 1
RCAD	Renal cysts and diabetes syndrome

N.	Sample number	Age at diagnosis / Relatives	Parental renal US	Renal phenotype	Hepatic phenotype	Extra-renal/hepatic phenotype	Clinical diagnosis	Genetic diagnosis	Detected sequence variants					
									Gene	DNA	Protein	dbSNP	ACMG	M/P
1	885/12	Neonatal	Normal	Enlarged kidneys with multiple cysts	Hepatomegaly, periportal fibrosis (US)	Congenital oculomotor apraxia	ARPKD	NPHP11	**TMEM67**	**c.1843T>C**	**p.Cys615Arg**	**rs201893408**	Likely Pathogenic	**P**
									TMEM67	**c.1843T>C**	**p.Cys615Arg**	**rs201893408**	Likely Pathogenic	**M**
	1181/13	healthy father							**TMEM67**	**c.1843T>C**	**p.Cys615Arg**	**rs201893408**		
	1182/13	healthy mother							**TMEM67**	**c.1843T>C**	**p.Cys615Arg**	**rs201893408**		
2	884/12	Neonatal	Normal	Enlarged kidneys with multiple cysts	Normal	No	ADPKD – VEO	Unknown	*PKD1*	*c.6965C>T*	*p.Thr2322Met*	*rs564570407*	*Benign*	---
									NEK8	*c.133C>T*	*p.Arg45Trp*	*rs1567759130*	*Uncertain Significance*	*De novo*
									COQ8B	*c.767C>A*	*Ala256Glu*	*rs201827222*	*Likely Benign*	*M*
	1177/13	healthy father												
	1178/13	healthy mother							*COQ8B*	*c.767C>A*	*Ala256Glu*			
3	1567/12	Perinatal	Normal	Massively enlarged kidneys (nephrectomy of right kidney)	Caroli´s syndrome	Respiratory insufficiency (resection of left upper pulmonary lobe)	ARPKD	ARPKD	**PKHD1**	**c.983G>A**	**p.Arg328Gln**	**rs770494581**	Likely Pathogenic	**P**
									PKHD1	**c.8114delG**	**p.Gly2705ValfsTer11**	**rs774050795**	Pathogenic	**M**
	129/13	healthy father							PKHD1	c.983G>A	p.Arg328Gln			
	128/13	healthy mother							PKHD1	c.8114delG	p.Gly2705ValfsTer11			
	249/19	maternal half-brother							PKHD1	c.8114delG	p.Gly2705ValfsTer11			
4	600/13	Prenatal/ Perinatal death	Normal	Enlarged kidneys with multiple cysts	Congenital hepatic fibrosis	Anhydramnios, pulmonary hypoplasia, anuria	ARPKD	ARPKD	**PKHD1**	**c.8792A>C**	**p.His2931Pro**	**N/A**	Uncertain Significance	**M**
									PKHD1	**Deletion of exon 62**	**N/A**	**N/A**	**N/A**	**P**
	601/13	healthy father							PKHD1	Deletion of exon 62				
	602/13	healthy mother							PKHD1	c.8792A>C	p.His2931Pro			
	603/13	healthy sister							PKHD1	Deletion of exon 62				
	582/14	third pregnancy							PKHD1	c.8792A>C	p.His2931Pro			
	443/17	maternal aunt							PKHD1	c.8792A>C	p.His2931Pro			
	99348/17	maternal aunt's partner							---	---	---			
5	893/13	Infantile (6 months)	Normal	Enlarged kidneys with multiple cysts	Hepatomegaly, periportal fibrosis (US)	No	ARPKD	ARPKD	**PKHD1**	**c.5895dupA**	**p.Leu1966ThrfsTer4**	**rs746838237**	Pathogenic	**P**
									PKHD1	**c.8114delG**	**p.Gly2705ValfsTer11**	**rs774050795**	Pathogenic	**M**
	895/13	healthy father							PKHD1	c.5895dupA	p.Leu1966ThrfsTer4			
	894/13	healthy mother							PKHD1	c.8114delG	p.Gly2705ValfsTer11			

6	1358/12	Neonatal	Normal	Enlarged kidneys with multiple cysts	Hepatomegaly, periportal fibrosis (US)	No	ARPKD	ARPKD	**PKHD1**	**c.107C>T**	**p.Thr36Met**	**rs137852944**	Likely Pathogenic	**M**
									PKHD1	**c.7561_7568delGCAGCAAT**	**p.Ala2521PhefsTer60**	**N/A**	Pathogenic	**---**
	949/14	healthy mother							PKHD1	c.107C>T	p.Thr36Met			
7	1177/12	Neonatal (anhydramnion)	Normal	Enlarged kidneys with multiple cysts	Normal (US)	No	ARPKD	ARPKD	**PKHD1**	**c.107C>T**	**p.Thr36Met**	**rs137852944**	Likely Pathogenic	**M**
									PKHD1	**c.10658T>C**	**p.Ile3553Thr**	**rs137852948**	Likely Pathogenic	**P**
	1075/14	healthy father							PKHD1	c.10658T>C	p.Ile3553Thr			
	1074/14	healthy mother							PKHD1	c.107C>T	p.Thr36Met			
8	1388/12	Prenatal/ Perinatal death	Normal	Enlarged kidneys with multiple cysts	Congenital hepatic fibrosis	Oligohydramnios, pulmonary hypoplasia	ARPKD	ARPKD	**PKHD1**	**c.107C>T**	**p.Thr36Met**	**rs137852944**	Likely Pathogenic	**P**
									PKHD1	**c.2309delG**	**p.Gly770AspfsTer7**	**N/A**	Likely Pathogenic	**M**
	019/13	healthy father							PKHD1	c.107C>T	p.Thr36Met			
	020/14	healthy mother							PKHD1	c.2309delG	p.Gly770AspfsTer7			
	239/18	healthy paternal aunt							---	---	---			
9	1479/12	Childhood (15 months)	Normal	Enlarged kidneys with multiple cysts and calcifications	Hepatosplenomegaly, CHF (biopsy proven), esophageal varices	No	ARPKD	Unknown	**PKHD1**	**c.8114delG**	**p.Gly2705ValfsTer11**	**rs774050795**	Pathogenic	**P**
									---	---	---	---	---	---
	012/14	healthy father							PKHD1	c.8114delG	p.Gly2705ValfsTer11			
	013/14	healthy mother							---	---	---			
10	446/13	Prenatal (TOP)	Normal	Bilaterally massively enlarged kidneys	Normal	Oligohydramnios	ARPKD	Unknown	**PKHD1**	**c.2713C>T**	**p.Gln905Ter**	**N/A**	Pathogenic	**M**
									PKD1	*c.8359C>T*	*p.Arg2787Cys*	*rs371765908*	*Likely Benign*	*P*
	960/13	healthy father							*PKD1*	*c.8359C>T*	*p.Arg2787Cys*			
	961/13	healthy mother							PKHD1	c.2713C>T	p.Gln905Ter			
11	1513/13	Perinatal/ Perinatal death	Normal	Massively enlarged kidneys	Caroli´s syndrome	Pulmonary hypoplasia	ARPKD	ARPKD	**PKHD1**	**c.107C>T**	**p.Thr36Met**	**rs137852944**	Likely Pathogenic	**M**
									PKHD1	**c.8011C>T**	**p.Arg2671Ter**	**rs137852947**	Pathogenic	**De novo**
	1512/13	healthy father							---	---	---			
	1511/13	healthy mother							PKHD1	c.107C>T	p.Thr36Met			
	1139/14	second pregnancy							---	---	---			
	988/14	maternal grandmother							---	---	---			
	1234/14	maternal grandfather							PKHD1	c.107C>T	p.Thr36Met			
	989/14	maternal aunt							PKHD1	c.107C>T	p.Thr36Met			

12	1359/12	Neonatal (US screening)	Normal	Enlarged kidneys with multiple cysts	Hepatomegaly, periportal fibrosis (US)	No	ARPKD	ARPKD	**PKHD1**	**c.4870C>T**	**p.Arg1624Trp**	**rs200391019**	Uncertain Significance	**P**
									PKHD1	**c.5323C>T**	**p.Arg1775Ter**	**rs770522674**	Pathogenic	**M**
	1180/13	healthy father							PKHD1	c.4870C>T	p.Arg1624Trp			
	1179/13	healthy mother							PKHD1	c.5323C>T	p.Arg1775Ter			
13	889/13	Prenatal	Normal	Enlarged kidneys with multiple cysts	Normal	No	ARPKD	NPHP11	**TMEM67**	**c.1843T>C**	**p.Cys615Arg**	**rs201893408**	Likely Pathogenic	**P**
									TMEM67	**c.1843T>C**	**p.Cys615Arg**	**rs201893408**	Likely Pathogenic	**M**
	890/13	healthy father							TMEM67	c.1843T>C	p.Cys615Arg			
	891/13	healthy mother							TMEM67	c.1843T>C	p.Cys615Arg			
	1295/14	sister with the same diagnosis							TMEM67	c.1843T>C	p.Cys615Arg			
									TMEM67	c.1843T>C	p.Cys615Arg			
14	974/13	Prenatal (TOP)	Normal	Hypoplastic kidneys with cystically dilated collecting ducts	Normal	Anhydramnios, pes equinovarus, Potter facies	PKD of unknown etiology	Unknown	DNA of proband was of low concentration and quality, DNA of proband's parents was used for the analysis.					
	832/13	healthy father							---	---	---	---	---	---
	891/13	healthy mother							---	---	---	---	---	---
15	91192/13	Prenatal (TOP)	Mother with ADPKD (PKD2 mutation)	Bilaterally massively enlarged kidneys	Normal	Oligohydramnios, pulmonary hypoplasia	ARPKD, familial ADPKD	Unknown (ADPKD) Sample of the fetus was analyzed for PKHD1 mutations as severe prognosis was reported in the fetus.	---	---	---	---	---	---
16	124/14	Childhood (3 years)	No	Massively enlarged kidneys with bilateral cortical microcysts, calicolithiasis, nephrocalcinosis	At the age of 3 probable congenital hepatic fibrosis	Splenomegaly, hypercalciuria, heart atrial defect type II, epileptic attack	ARPKD, NPHP	ARPKD	**PKHD1**	**c.4870C>T**	**p.Arg1624Trp**	**rs200391019**	Uncertain Significance	**Non paternity**
									PKHD1	**c.5895dupA**	**p.Leu1966ThrfsTer4**	**rs746838237**	Pathogenic	**M**
	1088/14	healthy father							---	---	---			
	1089/14	healthy mother							PKHD1	c.5895dupA	p.Leu1966ThrfsTer4			

	1373/14	healthy brother							PKHD1	c.5895dupA	p.Leu1966ThrfsTer4			
	1479/14	healthy maternal half-brother							---	---	---			
17	461/14	Prenatal	Normal	Polycystic kidneys	Normal	Oligohydramnios, right-sided pneumothorax after birth, hyponatremia	ARPKD, HNF1B	ARPKD	**PKHD1**	**c.664A>G**	**p.Ile222Val**	**rs369925690**	Likely Pathogenic	**P**
									PKHD1	**c.2264C>T**	**p.Pro755Leu**	**rs1057517158**	Pathogenic	**M**
18	460/14	Childhood (6 years)	Normal	Enlarged kidneys with bilaterally hyperechogenic cortex, small cysts	Normal	Oligohydramnios	ARPKD, HNF1B	ARPKD	**PKHD1**	**c.664A>G**	**p.Ile222Val**	**rs369925690**	Likely Pathogenic	**P**
									PKHD1	**c.2264C>T**	**p.Pro755Leu**	**rs1057517158**	Pathogenic	**M**
	459/14	healthy father							PKHD1	c.664A>G	p.Ile222Val			
	458/14	healthy mother							PKHD1	c.2264C>T	p.Pro755Leu			
19	1340/13	Neonatal (polyhydramnios, RDS, disproportional growth)	Normal	Enlarged kidneys with increased echogenity	Normal	Enteral polyps	ARPKD	Unknown (later reported that proband has bilateral blastema nephroblastoma)	*KIF7*	*c.2227C>T*	*p.Gln743Ter*	*N/A*	*Pathogenic*	---
									GLIS2	*c.737G>A*	*p.Arg246His*	*rs770824489*	*Uncertain Significance*	---
20	1371/13	Infantile (6 months)	Mother with ADPKD	Normal sized kidneys with multiple cysts	Hepatomegaly, multinodular hepatic cirrhosis (biopsy)	Insulin resistance, obesity	ADPKD	ADPKD	**PKD1**	**c.5653dupG**	**p.Glu1885GlyfsTer105**	**N/A**	Pathogenic	**M**
		mother with ADPKD							PKD1	c.5653dupG	p.Glu1885GlyfsTer105			
21	605/14	Infantile (2 months)	mother normal, father refuses	Enlarged kidneys with multiple cysts, without tubule dilation, loss of corticomedullary differentiation	Congenital hepatic fibrosis	Low levels of Insulin-like growth factor 1 - growth hormone treatment, latent iron deficiency, hypothyroidism	ARPKD	Unknown	**PKHD1**	**c.107C>T**	**p.Thr36Met**	**rs137852944**	Likely Pathogenic	**M**
									---	---	---	---	---	---
	1426/14	healthy father							---	---	---			
	1555/14	healthy mother							PKHD1	c.107C>T	p.Thr36Met			
	654/16	healthy brother							---	---	---			

22	883/14	Prenatal	Normal	Enlarged bilaterally hyperechogenic kidneys	Normal	Echogenic intracardiac focus	ADPKD, ARPKD	Unknown	---	---	---	---	---	---
23	1085/14	Perinatal	Normal	Bilaterally polycystic kidneys	Liver cysts (US)	Right-sided pneumothorax, mesocephaly, hyponatremia	ARPKD	ARPKD	**PKHD1**	**c.5895dupA**	**p.Leu1966ThrfsTer4**	**rs746838237**	Pathogenic	**P**
									PKHD1	**c.8114delG**	**p.Gly2705ValfsTer11**	**rs774050795**	Pathogenic	**M**
	1084/14	healthy father							PKHD1	c.5895dupA	p.Leu1966ThrfsTer4			
	1086/14	healthy mother							PKHD1	c.8114delG	p.Gly2705ValfsTer11			
24	943/12	Infantile (1 year)	Normal	Small sized hyperechogenic kidneys with several cysts	Normal sized liver with periportal fibrosis (US)	Aplasia of vagina and uterus, primary amenorrhea	ARPKD	Unknown (The Rokitansky-Kuster-Hauser syndrome later detected in the patient)	---	---	---	---	---	---
25	797/13	Neonatal	Normal	Renal pelvis dilatation, increasing number of cortical cysts	Not Disclosed	No	ARPKD	Unknown	**NPHP4**	**c.2882G>A**	**p.Arg961His**	**rs183885357**	Benign	**M**
	798/14	younger sister with the same diagnosis							NPHP4	c.2882G>A	p.Arg961His			
26	701/14	Neonatal	Normal	Hypoplastic kidneys with multiple cysts bilaterally	Normal	No	PKD, ARPKD	RCAD syndrome (results reported from another laboratory)	---	---	---	---	---	---
27	1052/14	Prenatal (TOP)	Normal	Enlarged kidneys with multiple small cysts	Normal	Anhydramnios	ARPKD	ARPKD	**PKHD1**	**c.107C>T**	**p.Thr36Met**	**rs137852944**	Likely Pathogenic	**M**
									PKHD1	**c.370C>T**	**p.Arg124Ter**	**rs727504096**	Pathogenic	**P**
	958/14	healthy father							PKHD1	c.370C>T	p.Arg124Ter			
	1051/14	healthy mother							PKHD1	c.107C>T	p.Thr36Met			
	508/15	Second pregnancy							---	---	---			
	810/18	third pregnancy							PKHD1	c.370C>T	p.Arg124Ter			

28	1006/14	Childhood (8 years)	Normal	Not disclosed/ after RRT	Normal	No	ARPKD	ARPKD	**PKHD1**	**c.2810G>A**	**p.Trp937Ter**	**rs786204707**	Pathogenic	**M**
									PKHD1	**c.4870C>T**	**p.Arg1624Trp**	**rs200391019**	Uncertain Significance	**? father dead**
	129/15	healthy mother							PKHD1	c.2810G>A	p.Trp937Ter			
	154/12	healthy partner							---	---	---			
29	1178/12	Neonatal (preterm delivery, 29th gest. week, posthemorrhagic hydrocephalus)	Normal renal US (mother uterus duplex)	Normal sized hyperechogenic kidneys	Normal	Psychomotor retardation	RCAD syndrome	RCAD syndrome	**HNF1B**	**Whole gene deletion**	**N/A**	**N/A**	**N/A**	**M**
30	886/12	Infantile	Normal	Enlarged hyperechogenic kidneys with multiple cysts and calcifications	Normal	No	ARPKD	Unknown	**PKHD1**	**c.920T>C**	**p.Ile307Thr**	**rs1288017883**	Likely Pathogenic	**---**
									ACTN4	*c.1279G>A*	*p.Ala427Thr*	*rs201128110*	*Benign*	---
31	1360/12	Neonatal	Normal	Enlarged hyperechogenic kidneys	Normal	Oligohydramnios	RCAD syndrome	RCAD syndrome (results reported from another laboratory)	---	---	---	---	---	---
32	887/12	Neonatal	Normal	Normal sized hyperechogenic kidneys	Hepatomegaly, periportal fibrosis (US)	Nystagmus	ARPKD	NPHP11	**TMEM67**	**c.1843T>C**	**p.Cys615Arg**	**rs201893408**	Likely Pathogenic	**In trans**
									TMEM67	**c.1815_1831delGGAAGAACGTTTTGTCA**	**p.Gln605HisfsTer17**	**N/A**	Pathogenic	**In trans**
33	141/15 FFPE	Not disclosed (prenatal - childhood, death at 5 years of age)	Normal	Enlarged hyperechogenic kidneys	Hepatosplenomegaly, multinodular hepatic cirrhosis, esophageal varices	Pulmonary hypoplasia, death at 5 years of age (septic shock)	ARPKD	probably ARPKD	DNA of proband was of low concentration and quality, DNA of proband's parents was used for the analysis.					
	078/15	healthy father							**PKHD1**	**c.107C>T**	**p.Thr36Met**	**rs137852944**	Likely Pathogenic	
	079/15	healthy mother							**PKHD1**	**c.107C>T**	**p.Thr36Met**	**rs137852944**	Likely Pathogenic	

34	160/15	Prenatal	Normal	Enlarged kidneys, bilateral polyectasis	Normal	Polyhydramnios, fetal megavesica, suspected lethal congenital contracture syndrome (ruled after the birth), arachnodactyly, hyponatremia, hypomagnesemia, hypocalcemia, hypophosphatemia	ARPKD	Unknown	*COL4A3*	*c.3829G>A*	*p.Gly1277Ser*	*rs190598500*	*Likely Pathogenic*	---
35	888/12	Infantile (3 months)	Normal	Enlarged hyperechogenic kidneys	Normal sized liver with periportal fibrosis (US)	No	ARPKD	ARPKD	**PKHD1**	**c.107C>T**	**p.Thr36Met**	**rs137852944**	Likely Pathogenic	**P**
									PKHD1	**c.8114delG**	**p.Gly2705ValfsTer11**	**rs774050795**	Pathogenic	**M**
	1572/15	healthy father							PKHD1	c.107C>T	p.Thr36Met			
	630/16	healthy mother							PKHD1	c.8114delG	p.Gly2705ValfsTer11			
36	865/12	Neonatal	Normal	Enlarged kidneys with multiple cysts	Hepatosplenomegaly, congenital hepatic fibrosis (US)	No	ARPKD	ARPKD	**PKHD1**	**c.2725C>T**	**p.Arg909Ter**	**rs727504089**	Pathogenic	**?**
									PKHD1	**c.8870T>C**	**p.Ile2957Thr**	**rs760222236**	Likely Pathogenic	**M**
	1346/15	healthy mother							PKHD1	c.8870T>C	p.Ile2957Thr			
37	863/12	Neonatal	Normal	Enlarged kidneys with multiple cysts	Hepatomegaly, periportal fibrosis with cysts (US)	No	ARPKD	ARPKD	**PKHD1**	**c.4403T>C**	**p.Leu1468Pro**	**rs140331370**	Likely Pathogenic	**P**
									PKHD1	**c.8870T>C**	**p.Ile2957Thr**	**rs760222236**	Likely Pathogenic	**M**
	842/15	healthy father							PKHD1	c.4403T>C	p.Leu1468Pro			
	843/15	healthy mother							PKHD1	c.8870T>C	p.Ile2957Thr			
38	883/12	Neonatal (anhydramnios)	Normal	Enlarged kidneys with multiple cysts	Hepatomegaly, periportal fibrosis with cysts (US)	Mild mental retardation	ARPKD	ARPKD	**PKHD1**	**c.5323C>T**	**p.Arg1775Ter**	**rs770522674**	Pathogenic	**M**
									PKHD1	**c.5060T>C**	**p.Ile1687Thr**	**rs794727566**	Pathogenic	**P**
	43/16	healthy father							PKHD1	c.5060T>C	p.Ile1687Thr			
	128/16	healthy mother							PKHD1	c.5323C>T	p.Arg1775Ter			
39	765/12	Neonatal	Normal	Enlarged kidneys with multiple cysts	Hepatosplenomegaly, CHF (US), esophageal varices	No	ARPKD	Unknown	**PKHD1**	**c.107C>T**	**p.Thr36Met**	**rs137852944**	Likely Pathogenic	---
									ZNF423	*c.964T>C*	*p.Ser322Pro*	*rs142835239*	*Likely Benign*	---
40	889/12	Infantile (4 months)	Normal	Hyperechogenic kidneys	Normal sized with irregular structure (US)	No	ARPKD	NPHP11	**TMEM67**	**c.1843T>C**	**p.Cys615Arg**	**rs201893408**	Likely Pathogenic	**---**
									TMEM67	**c.1843T>C**	**p.Cys615Arg**	**rs201893408**	Likely Pathogenic	**---**
41	864/12	Neonatal	Normal	Enlarged kidneys with multiple cysts	Hepatosplenomegaly, congenital hepatic fibrosis (US), oesofageal varices	No	ARPKD	ARPKD	**PKHD1**	**c.107C>T**	**p.Thr36Met**	**rs137852944**	Likely Pathogenic	**P**
									PKHD1	**c.9719G>A**	**p.Arg3240Gln**	**rs146649803**	Likely Pathogenic	**M**
	674/15	healthy father							PKHD1	c.107C>T	p.Thr36Met			
	675/15	healthy mother							PKHD1	c.9719G>A	p.Arg3240Gln			

42	637/15	Infantile	Normal	Not disclosed	Not disclosed	Not disclosed	ARPKD	ARPKD	**PKHD1**	**c.8114delG**	**p.Gly2705ValfsTer11**	**rs774050795**	Pathogenic	**P**
									PKHD1	**c.8114delG**	**p.Gly2705ValfsTer11**	**rs774050795**	Pathogenic	**M**
	636/15	healthy father							PKHD1	c.8114delG	p.Gly2705ValfsTer11			
	635/15	healthy mother							PKHD1	c.8114delG	p.Gly2705ValfsTer11			
43	640/15	Infantile (2 months)	Normal	Bilaterally enlarged kidneys with cysts	Normal sized with irregular structure (US)	Hyponatremia, secondary hyperuricemia, renal anemia	ARPKD	Unknown	**PKHD1**	**c.8114delG**	**p.Gly2705ValfsTer11**	**rs774050795**	Pathogenic	**P**
									NPHP3	*Suspected deletion of exons 14-16*	*N/A*	*N/A*	*N/A*	*?*
	638/15	healthy father							PKHD1	c.8114delG	p.Gly2705ValfsTer11			
	639/15	healthy mother												
44	378/15	Childhood (2 years)	Normal	Bilaterally enlarged kidneys with cysts	Hepatosplenomegaly, Caroli's disease	No	ARPKD	ARPKD	**PKHD1**	**c.100G>A**	**p.Gly34Arg**	**N/A**	Uncertain Significance	**M**
									PKHD1	**c.664A>G**	**p.Ile222Val**	**rs369925690**	Likely Pathogenic	**P**
	379/15	healthy father							PKHD1	c.664A>G	p.Ile222Val			
	377/15	healthy mother							PKHD1	c.100G>A	p.Gly34Arg			
45	468/15	prenatal (TOP) First pregnancy	Normal	Multicystic dysplastic kidneys	Not Disclosed	Not Disclosed	PKD of unknown etiology, ARPKD	Unknown	DNA of proband was of low concentration and quality, DNA of proband's parents was used for the analysis.					
									PKHD1	*c.275G>A*	*p.Arg92Gln*			
	471/15	healthy father							*PKHD1*	*c.275G>A*	*p.Arg92Gln*	*rs145886657*	Uncertain Significance	---
	467/15	healthy mother							*PKHD1*	*c.1249A>G*	*p.Ile417Val*	*rs781286941*	Uncertain Significance	---
	469/15	prenatal (TOP) Second pregnancy	Normal	Multicystic dysplastic kidneys	Not Disclosed	Not Disclosed	PKD of unknown etiology, ARPKD	Unknown	*PKHD1*	*c.275G>A*	*p.Arg92Gln*			
									PKHD1	*c.1249A>G*	*p.Ile417Val*			
46	642/15	Childhood (10 years)	Normal	Bilaterally polycystic kidneys	Normal	No	PKD of unknown etiology	Unknown	*PKHD1*	*c.6784A>T*	*p.Ile2262Phe*	*N/A*	Uncertain Significance	---
47	1179/14	Prenatal (TOP)	Normal	Cystic dysplastic kidneys	Normal	Oligohydramnios	ARPKD	Unknown	*PKHD1*	*c.7673G>C*	*p.Arg2558Pro*	*rs369677008*	Uncertain Significance	---
48	589/15	Childhood (4 years)	Normal	Normal	Congenital hepatic fibrosis, esophageal varices, splenomegaly	No	Unknown diagnosis with phenotype of congenital hepatic fibrosis (ARPKD?)	Unknown	---	---	---	---	---	---

49	1011/15	Childhood (4 years)	Normal	Hyperechogenic kidneys with nephrocalcinosis	Hepatosplenomegaly, congenital hepatic fibrosis	Hypertrophic cardiomyopathy	ARPKD, HNF1B	ARPKD	**PKHD1**	**c.107C>T**	**p.Thr36Met**	**rs137852944**	Likely Pathogenic	**M**
									PKHD1	**c.3407A>G**	**p.Tyr1136Cys**	**rs41273726**	Benign	**P**
	302/16	healthy father							PKHD1	c.3407A>G	p.Tyr1136Cys			
	301/16	healthy mother							PKHD1	c.107C>T	p.Thr36Met			
	929/16	healthy maternal aunt							---	---	---			
	930/16	healthy brother							---	---	---			
50	1294/15	Neonatal	Normal	Bilaterally enlarged polycystic kidneys	Liver cysts	Oligohydramnios, hypotonia after birth, mesocephaly, clinodactyly, equinus deformity of left foot	ARPKD	ARPKD	**PKHD1**	**c.107C>T**	**p.Thr36Met**	**rs137852944**	Likely Pathogenic	**M**
									PKHD1	**c.9146A>G**	**p.His3049Arg**	**rs367678592**	Uncertain Significance	**P**
	375/16	healthy father							PKHD1	c.9146A>G	p.His3049Arg			
	374/16	healthy mother							PKHD1	c.107C>T	p.Thr36Met			
	1039/16	healthy paternal aunt							PKHD1	c.9146A>G	p.His3049Arg			
	1167/16	healthy paternal cousin							PKHD1	c.9146A>G	p.His3049Arg			
51	1357/15	prenatal (TOP) first pregnancy	Normal	Bilateral multicystic dysplastic kidney	Congenital hepatic fibrosis	Equinus deformity of left foot	ARPKD	ARPKD	DNA of proband was of low concentration and quality, DNA of proband's parents was used for the analysis.					
									PKHD1	c.9737C>T	p.Pro3246Leu			
									PKHD1	c.2341C>T	p.Arg781Ter			
	1291/15	healthy father							**PKHD1**	**c.9737C>T**	**p.Pro3246Leu**	**N/A**	Uncertain Significance	**---**
	1290/15	healthy mother							**PKHD1**	**c.2341C>T**	**p.Arg781Ter**	**rs398124478**	Pathogenic	**---**
	569/16	second pregnancy							---	---	---			
52	094/16	Infantile (10 months)	Normal	Normal sized hyperechogenic kidneys	Hepatomegaly, periportal fibrosis (US)	No	ARPKD	NPHP11 (results reported from commercial laboratory)	---	---	---	---	---	---
53	021/16	Infantile	Normal	Not disclosed	Not disclosed	Not disclosed	ARPKD	ARPKD	**PKHD1**	**c.10414T>C**	**p.Cys3472Arg**	**N/A**	Likely Pathogenic	**P**
									PKHD1	**c.2408-2A>G**	**N/A**	**rs1554210085**	Pathogenic	**M**
	795/16	healthy father							PKHD1	c.10414T>C	p.Cys3472Arg			
	796/16	healthy mother							PKHD1	c.2408-2A>G	N/A			

54	1439/15	Prenatal	Normal	Bilaterally hyperechogenic kidneys with micro and macrocysts	Not disclosed	No	PKD of unknown etiology, ARPKD	RCAD syndrome	**De novo HNF1B mutation found within another project**						
	703/16	healthy father							---	---	---				
	1440/15	healthy mother							---	---	---				
55	194/16	Adulthood (incidental finding at 31 years)	Normal	Normal sized kidneys with cysts bilaterally	Normal	No	PKD of unknown etiology	ARPKD	**PKHD1**	**c.5895dupA**	**p.Leu1966ThrfsTer4**	**rs746838237**	Pathogenic	---	
									PKHD1	**c.5830G>A**	**p.Asp1944Asn**	**rs774290802**	Likely Pathogenic	---	
	1561/18	son							PKHD1	c.5830G>A	p.Asp1944Asn				
	1562/18	son							PKHD1	c.5830G>A	p.Asp1944Asn				
56	1040/16	Childhood (12 years)	Normal	Normal sized kidneys with cysts	Normal	Polydactyly, obesity, learning difficulties	BBS	BBS1	**BBS1**	**c.46_47delAG**	**p.Ser16GlnfsTer2**	**rs1291184039**	Pathogenic	**P**	
									BBS1	**c.1660_1661delAG**	**p.Leu555GlnfsTer2**	**rs1209299063**	Pathogenic	**M**	
	1239/18	healthy father							BBS1	c.46_47delAG	p.Ser16GlnfsTer2				
	1240/18	healthy mother							BBS1	c.1660_1661delAG	p.Leu555GlnfsTer2				
	1421/18	healthy brother							---	---	---				
57	001/16	Adulthood	Normal	Enlarged kidneys with multiple cysts	Normal	Normal	ADPKD	Unknown	---	---	---	---	---	---	
58	282/16	Adulthood	Family history of ADPKD	Polycystic kidneys	Normal	No	ADPKD	ADPKD	**PKD1**	**c.8311G>A**	**p.Glu2771Lys**	**rs1057518897**	Likely Pathogenic	---	
	241/11	healthy grandmother							---	---	---				
	199/11	healthy mother							---	---	---				
	189/11	healthy sister							---	---	---				
	401/08	brother with ADPKD							PKD1	c.8311G>A	p.Glu2771Lys				
	397/08	healthy daughter							---	---	---				
	399/08 (037/11)	daughter with ADPKD							PKD1	c.8311G>A	p.Glu2771Lys				
59	69/14	Adulthood	Family history of ADPKD	Not disclosed	Not disclosed	Not disclosed	ADPKD	ADPKD	PKD1	c.11379delG	p.Thr3794ArgfsTer32	rs1567153758	Pathogenic	---	
	105/18	First pregnancy							PKD1	c.11379delG	p.Thr3794ArgfsTer32				
60	383/16	Not disclosed (prenatal - infantile)	Not disclosed	Not disclosed	Normal	Cleft palate, milias, brachydactyly, micrognation,	OFD1	OFD1	**OFD1**	**c.1358_1362delTACTT**	**p.Leu453Ter**	**N/A**	Pathogenic	---	

						hypertelorism, antimongoloid slant, high-arched palate, psychomotor retardation								
	1334/16	healthy mother							---	---	---			
61	472/16	Perinatal/ Perinatal death	Normal	Enlarged kidneys with microcysts	Not disclosed	Pulmonary hypoplasia, respiratory distress syndrome, pneumothorax	Suspected NPHP	Unknown	*WT1*	*c.1315C>T*	*p.Arg439Cys*	*rs121907910*	Likely Pathogenic	*De novo*
	474/16	healthy father							---	---	---			
	473/16	healthy mother							---	---	---			
62	407/16	Childhood	Normal	Kidneys with multiple cysts, hydronephrosis, megaureter	Normal	No	PKD of unknown etiology	Unknown	---	---	---	---	---	---
63	515/15	Childhood (5 years)	Normal	Normal sized kidneys with cysts (disappearance of cysts in one kidney during follow-up)	Normal	Encapsulated abdominal hematoma (extirpated)	PKD of unknown etiology	Unknown	**PKHD1**	**c.8870T>C**	**p.Ile2957Thr**	**rs760222236**	Likely Pathogenic	**P**
									TMEM237	*c.52C>T*	*p.Arg18Ter*	*rs199469707*	*Pathogenic*	*M*
	518/15	healthy father							PKHD1	c.8870T>C	p.Ile2957Thr			
	517/15	healthy mother							TMEM237	c.52C>T	p.Arg18Ter			
	516/15	healthy sister							---	---	---			
64	419/17	Neonatal	Normal	Bilaterally enlarged hyperechogenic kidneys	Normal	No	ARPKD, HNF1B	NPHP11	**TMEM67**	**c.1843T>C**	**p.Cys615Arg**	**rs201893408**	Likely Pathogenic	**M**
									TMEM67	**c.1843T>C**	**p.Cys615Arg**	**rs201893408**	Likely Pathogenic	**P**
	952/18	healthy father							TMEM67	c.1843T>C	p.Cys615Arg			
	953/18	healthy mother							TMEM67	c.1843T>C	p.Cys615Arg			
65	859/16	Childhood (8 years)	Normal	Multiple small cyst bilaterally	Normal	No	PKD of unknown etiology	Unknown	**TMEM67**	**c.653G>C**	**p.Gly218Ala**	**rs202036490**	Uncertain Significance	**M**
									---	---	---	---	---	---
	1187/18	healthy father							---	---	---			
	1188/18	healthy mother							TMEM67	c.653G>C	p.Gly218Ala			
66	952/16	Adulthood	Normal	Polycystic kidneys	Cysts in liver	No	ADPKD	ADPKD	**PKD1**	**c.11693C>A**	**p.Ser3898Ter**	**N/A**	Pathogenic	**De novo**
	1726/18	healthy father							---	---	---			
	1728/18	healthy mother							---	---	---			
	1729/18	healthy sister							---	---	---			
	1730/18	son							---	---	---			
	1727/18	son							---	---	---			

67	449/16	Childhood (2 years)	Normal	Normal sized kidneys with cysts	Normal	No	ADPKD-VEO	ADPKD	**PKD1**	**c.10619-2A>G**	**N/A**	**N/A**	Pathogenic	**De novo**
	518/18	healthy father							---	---	---			
	517/18	healthy mother							---	---	---			
68	236/16	Childhood (9 years)	Normal	Bilaterally polycystic kidneys	Normal	No	PKD of unknown etiology	Unknown (ADPKD)	**PKD1 mosaic (20%)**	**c.9158C>A**	**p.Ala3053Asp**	**N/A**	Uncertain Significance	**De novo**
	526/19	healthy father							---	---	---			
	525/19	healthy mother							---	---	---			
69	352/17	Prenatal (TOP)	Not done	Kidneys with multiple cysts	Normal	Oligo/anhydramnios	ARPKD	Unknown	---	---	---	---	---	---
70	770/17	Neonatal	Normal	Hyperechogenic kidneys with multiple cysts	Normal	Oligohydramnios, scrotal hypospadia, small penis, hydrocele, hyponatremia, hypertelorism etc.	PKD of unknown etiology	Unknown	*PKHD1*	*c.5410C>T*	*p.Arg1804Cys*	*rs201906247*	*Uncertain Significance*	*M*
									PKHD1	*c.1342G>C*	*p.Gly448Arg*	*rs149781976*	Uncertain Significance	*M*
	361/19	healthy mother							*PKHD1*	*c.5410C>T*	*p.Arg1804Cys*			
									PKHD1	*c.1342G>C*	*p.Gly448Arg*			
71	759/17	Prenatal (TOP)	Normal	Bilaterally multicystic dysplastic kidneys	Normal	Oligohydramnios	PKD of unknown etiology	Unknown	---	---	---	---	---	---
72	606/17	Neonatal	Normal	Enlarged kidneys with multiple cysts	Normal	Failure to thrive, speech delay, hypercalcemia, hypercalciuria	PKD of unknown etiology	Unknown	*PKD1*	*c.7124C>T*	*p.Ala2375Val*	*rs780145654*	*Benign*	*P*
	1230/18	healthy father							PKD1	c.7124C>T	p.Ala2375Val			
	1229/18	healthy mother							---	---	---			
	1228/18	healthy brother							---	---	---			
73	540/17	Adolescence (17 years)	Normal	Hyperechogenic kidneys with multiple cysts	Normal	No	ARPKD	ADPKD	**PKD1**	**c.8162-1G>C**	**N/A**	**N/A**	Pathogenic	**De novo**
74	541/17	Adolescence (15 years)	Normal	Hyperechogenic kidneys with multiple cysts	Normal	No	ARPKD	ADPKD	**PKD1**	**c.8162-1G>C**	**N/A**	**N/A**	Pathogenic	**De novo**
	543/17	healthy father							---	---	---			
	542/17	healthy mother							---	---	---			
75	1458/16	Prenatal (TOP)	Normal	Multicystic dysplastic kidneys	Normal	Face dysmorphia, pulmonary hypoplasia, postaxial polydactyly, hypertelorism	BBS / Meckel syndrome	BBS10	**BBS10**	**c.1804G>C**	**p.Val602Leu**	**rs778431173**	Pathogenic	**P**
									BBS10	**c.271dupT**	**p.Cys91LeufsTer5**	**rs549625604**	Pathogenic	**M**
	1625/19	healthy father							BBS10	c.1804G>C	p.Val602Leu			
	1626/19	healthy mother							BBS10	c.271dupT	p.Cys91LeufsTer5			

	1627/19	second pregnancy							BBS10	c.1804G>C	p.Val602Leu			
									BBS10	c.271dupT	p.Cys91LeufsTer5			
76	88/17	Prenatal	Mother with ADPKD	Bilaterally enlarged kidneys, one cortical cyst	Normal	Oligohydramnios, pes equinovarius	ADPKD	Unknown (Unbalanced translocation reported in father – possible effect on phenotype of the proband)	*PKD1*	*c.11957C>T*	*p.Ala3986Val*	*rs528213425*	*Benign*	*M*
									PKD1	*c.9499A>T*	*p.Ile3167Phe*	*rs139945204*	*Benign*	*M*
	85/17	mother with ADPKD without family history							PKD1	c.11957C>T	p.Ala3986Val			
									PKD1	c.9499A>T	p.Ile3167Phe			
77	853/17	Neonatal	Normal	Unilateral kidney with multiple cysts	Normal	No	PKD of unknown etiology	Unknown	---	---	---	---	---	---
78	1023/18	Adulthood	Normal	Not disclosed	Not disclosed	Not disclosed	PKD of unknown etiology, NPHP	Suspected Papilloreal syndrome	**PAX2**	**c.497-2A>G**	**N/A**	**N/A**	Pathogenic	**---**
79	1252/18	Adulthood	Renal cysts in family	Not disclosed	Not disclosed	Not disclosed	PKD of unknown etiology	Unknown	---	---	---	---	---	---
80	1238/18	Childhood (8 years)	Normal	Normal sized hyperechogenic kidneys with cysts	Normal	Autism	NPHP	NPHP1	**NPHP1**	**Whole gene deletion**	**N/A**	**N/A**	**N/A**	**---**
									NPHP1	**Whole gene deletion**	**N/A**	**N/A**	**N/A**	**---**
81	1563/18	Adulthood	Normal	Smaller kidneys with multiple cysts	Normal	Normal	PKD of unknown etiology	Unknown	*SIX2*	*c.722C>T*	*p.Pro241Leu*	*rs147806994*	*Likely Benign*	---
82	1728/18	Adulthood	Not disclosed	Microcystosis	Normal	No	PKD of unknown etiology	Unknown	---	---	---	---	---	---
83	1350/18	Prenatal	Normal	Microcystosis	Normal	Coloboma of the optic nerve, astigmatism, hyperopia, anisometropia	PKD of unknown etiology	Papilloreal syndrome	**PAX2**	**Suspected gene deletion (exon 7 to 11)**	**N/A**	**N/A**		**---**

84	1351/18	Infantile (6 months)	Normal	Hyperechogenic kidneys with microcysts	Normal	Atypic papil of optic nerve	PKD of unknown etiology	Papilloreal syndrome	**PAX2**	**Suspected gene deletion (exons 7 to 11)**	**N/A**	**N/A**		**---**
85	1737/18	Childhood (8 years)	Normal	Normal sized kidneys with cortical cysts	Normal	Pancreatic cysts	RCAD syndrome	Unknown	*RET*	*c.2372A>T*	*p.Tyr791Phe*	*rs77724903*	*Likely Benign*	---
86	1668/18	Childhood (3 years)	Normal	Normal sized kidneys with cortical cysts	Normal	No	ADPKD-VEO	Unknown	---	---	---	---	---	---
87	1287/18	Prenatal (TOP)	Not disclosed	Not disclosed	Not disclosed	Not disclosed	Meckel syndrome	Meckel syndrome	**TMEM67**	**c.1288+2T>A**	**N/A**	**N/A**	Pathogenic	**M**
									TMEM67	**c.2239C>G**	**p.Gln747Glu**	**rs764097983**	Uncertain Significance	**P**
	90128/18	healthy father							TMEM67	c.2239C>G	p.Gln747Glu			
	90129/18	healthy mother							TMEM67	c.1288+2T>A	N/A			
88	863/18	Prenatal (TOP)	Not disclosed	Multicystic dysplastic kidneys	Normal	Anhydramnios, cardiomegaly	ARPKD	Unknown	---	---	---	---	---	---
89	1048/17	Adulthood	Family history of ADPKD	---	---	---	ADPKD	ADPKD	**PKD1**	**c.6846C>A**	**p.Tyr2282Ter**	**N/A**	Pathogenic	**---**
									PKD1	**c.6791C>A**	**p.Ser2264Ter**	**N/A**	Pathogenic	**---**
									PKD1	**c.6451dupG**	**p.Val2151GlyfsTer24**	**N/A**	Pathogenic	**---**
90	1000/17	Prenatal	Normal	Bilaterally enlarged polycystic kidneys	Normal	No	TSC2/PKD1 contiguous gene syndrome	TSC2/PKD1 contiguous gene syndrome	**PKD1**	**Deletion (exons 35-46)**		**N/A**	**N/A**	**De novo**
									TSC2	**Deletion (exons 31-42)**		**N/A**	**N/A**	**De novo**
	999/17	healthy father							---	---	---			
	1001/17	healthy mother							---	---	---			
91	191/17	Adulthood	Family history of ADPKD	---	---	---	ADPKD	ADPKD	**PKD1**	**c.317T>C**	**p.Leu106Ser**	**N/A**	Uncertain Significance	**---**
92	535/17	Adulthood	Family history of ADPKD	---	---	---	ADPKD	ADPKD	**PKD1**	**c.12419G>A**	**p.Trp4140Ter**	**N/A**	Pathogenic	**---**
93	1500/17	Adulthood	Family history of ADPKD	---	---	---	ADPKD	ADPKD	**PKD1**	**c.5014_5015delAG**	**p.Arg1672GlyfsTer98**	**rs1555455457**	Pathogenic	**---**
94	1505/17	Adulthood	Family history of ADPKD	---	---	---	ADPKD	ADPKD	**PKD1**	**c.6960_6961insG**	**p.Ser2321GlufsTer99**	**N/A**	Pathogenic	**---**

95	16/18	Adulthood	Normal	---	---	---	ADPKD	Unknown	*PKD1*	*c.5911G>A*	*p.Val1971Met*	*rs140434415*	*Likely Benign*	---
96	1244/17	Adulthood	Normal	---	---	---	ADPKD	Unknown	---	---	---	---	---	---
97	1295/17	Adulthood	Family history of ADPKD	---	---	---	ADPKD	Unknown	---	---	---	---	---	---
98	1344/17	Adulthood	Family history of ADPKD	---	---	---	ADPKD	Unknown	---	---	---	---	---	---
99	1339/17	Adulthood	Family history of ADPKD	---	---	---	ADPKD	ADPKD	**PKD1**	**c.317T>C**	**p.Leu106Ser**	**N/A**	Uncertain Significance	**---**
100	1124/17	Adulthood	Family history of ADPKD	---	---	---	ADPKD	ADPKD	**PKD1**	**c.1512dupA**	**p.Ala505SerfsTer14**	**N/A**	Pathogenic	**---**
	1125/17	daughter with ADPKD							**PKD1**	**c.1512dupA**	**p.Ala505SerfsTer14**			
	589/17	daughter with ADPKD							**PKD1**	**c.1512dupA**	**p.Ala505SerfsTer14**			
	1123/17	granddaughter with ADPKD							**PKD1**	**c.1512dupA**	**p.Ala505SerfsTer14**			
	254/17	maternal half-sister							**PKD1**	**c.1512dupA**	**p.Ala505SerfsTer14**			
	518/17	healthy son of half-sister							---	---	---			
	520/17	father of the half-sister							---	---	---			
	519/17	healthy mother							---	---	---			
101	562/16	Adulthood	Father with ADPKD	---	---	---	ADPKD	Unknown	---	---	---	---	---	---
102	497/18	Adulthood	Not disclosed	Not disclosed	Not disclosed	Not disclosed	ADPKD	Unknown	---	---	---	---	---	---
103	619/18	Adulthood	Mother with ADPKD	Polycystic kidneys	Normal	Recurrent miscarriages	ADPKD	ADPKD	**PKD1**	**c.10995delG**	**p.Arg3666GlyfsTer18**	**N/A**	Pathogenic	**M**
	620/18	mother with ADPKD							PKD1	c.10995delG	p.Arg3666GlyfsTer18			
104	1184/18	Childhood (5 years)	Normal	Normal sized kidneys with cysts	Normal	No	ARPKD	ADPKD (de novo)	**PKD1**	**c.6090delC**	**p.Val2031TrpfsTer85**	**N/A**	Pathogenic	**---**
105		Childhood	Mother with ADPKD	Not disclosed	Not disclosed	Not disclosed	ADPKD	ADPKD	**PKD1**	**c.9897delC**	**p.Tyr3299Ter**	**N/A**	Likely Pathogenic	**M**
	1049/13	maternal grandmother with ADPKD							PKD1	c.9897delC	p.Tyr3299Ter			

	1050/13	mother with ADPKD							PKD1	c.9897delC	p.Tyr3299Ter			
106	1627/17	Adulthood	Mother with ADPKD	Not disclosed	Not disclosed	Not disclosed	ADPKD	ADPKD	**PKD1**	**c.5977_5987del**	**p.Thr1993AlafsTer53**	**N/A**	Pathogenic	**M**
	51/18	mother with ADPKD							**PKD1**	**c.5977_5987del**	**p.Thr1993AlafsTer53**			
	53/18	healthy sister							---	---	---			
	50/18	healthy father							---	---	---			
107	1490/18	Adulthood (hypertension at 25, PKD proved at 40))	Father with ADPKD	---	---	---	ADPKD	ADPKD	**PKD1**	**c.7546C>T**	**p.Arg2516Cys**	**rs797044902**	Likely Pathogenic	**---**
108	I.19	Adulthood	Father with ADPKD	---	---	---	ADPKD	ADPKD	**PKD1**	**c.11258G>A**	**p.Arg3753Gln**	**rs1555446330**	Likely Pathogenic	**--- father dead**
	IV.19	healthy mother							---	---	---			
	III.19	healthy daughter							---	---	---			
		daughter with ADPKD							PKD1	c.11258G>A	p.Arg3753Gln			
109	1724/18	Adulthood	Not disclosed	---	---	---	ADPKD	ADPKD	**PKD1**	**c.9240_9241delAT**	**p.Ala3082CysfsTer96**	**rs1567173924**	Pathogenic	**---**
	999/08	healthy brother							---	---	---			
	1001/08	niece							---	---	---			
	997/08	niece							---	---	---			
110	442/15	Adulthood	Father with ADPKD	---	---	Aneurysm	ADPKD	Unknown	---	---	---	---	---	---
111	775/15	Adulthood	Family history of ADPKD	---	---	---	ADPKD	Unknown	---	---	---	---	---	---
112	1345/18	Adulthood	Not disclosed	---	---	---	ADPKD	Unknown	---	---	---	---	---	---
113	696/19	Adulthood	Family history of ADPKD	---	---	---	ADPKD	ADPKD	**PKD1**	**c.11225delC**	**p.Pro3742GlnfsTer84**	**N/A**	Pathogenic	**---**
114	388/18	Adulthood	Family history of ADPKD	---	---	---	ADPKD	ADPKD	**PKD1**	**c.12683G>C**	**p.Arg4228Pro**	**rs1064797205**	Likely Pathogenic	**---**
115	312/19	Adulthood	Mother with ADPKD	---	---	---	ADPKD	ADPKD	**PKD1**	**c.7837_7839delTTG**	**p.Leu2613del**	**N/A**	Likely Pathogenic	**---**
116	715/19	Adulthood	Mother with ADPKD	---	---	---	ADPKD	ADPKD	**PKD1**	**c.7837_7839delTTG**	**p.Leu2613del**	**N/A**	Likely Pathogenic	**---**

117	862/19	Childhood (10 years)	Mother with ADPKD	---	---	Retention of testes, oligozoospermia	ADPKD	ADPKD	**PKD1**	**c.2528C>G**	**p.Ser843Ter**	**N/A**	Pathogenic	---
118	825/18	Adulthood	Mother with ADPKD	---	---	---	ADPKD	ADPKD	**PKD1**	**c.1141G>A**	**p.Gly381Ser**	**rs1303102528**	Uncertain Significance	---
119	882/19	Adulthood	Mother with ADPKD	RRT (52 years)	---	---	ADPKD	ADPKD	**PKD1**	**c.7915C>T**	**p.Arg2639Ter**	**N/A**	Pathogenic	---
120	1612/18	Adulthood	Not disclosed	---	---	---	ADPKD	ADPKD	**PKD1**	**c.160_166dupCGCGGGC**	**p.Leu56ProfsTer60**	**rs3072277**	Likely Pathogenic	---
121	31/19	Adulthood	Not disclosed	Normal sized kidneys with cysts	---	---	ADPKD	ADPKD	**PKD1**	**c.2534T>C**	**p.Leu845Ser**	**rs199476100**	Pathogenic	---
122	1155/17	Prenatal (TOP)		Bilaterally enlarged polycystic kidneys without corticomedullar differentiation	Normal	Oligohydramnios, hypertelorism, microstomy	ARPKD	ARPKD	**PKHD1**	**c.107C>T**	**p.Thr36Met**	**rs137852944**	Likely Pathogenic	**M**
									PKHD1	**c.7023T>A**	**p.Tyr2341Ter**	**N/A**	Pathogenic	**P**
	298/18	healthy father							PKHD1	c.7023T>A	p.Tyr2341Ter			
	297/18	healthy mother							PKHD1	c.107C>T	p.Thr36Met			
	441/18	second pregnancy							PKHD1	c.7023T>A	p.Tyr2341Ter			
	146/20	third pregnancy							PKHD1	c.107C>T	p.Thr36Met			
	1114/18	maternal aunt							PKHD1	c.107C>T	p.Thr36Met			
	1348/18	maternal aunt partner							---	---	---			
	1115/18	maternal aunt							---	---	---			
123	916/15	Neonatal	Father with ADPKD	Enlarged kidneys with multiple cysts	Normal	No	ADPKD-VEO	ADPKD	**PKD1**	**c.12442G>T**	**p.Glu4148Ter**	**N/A**	Pathogenic	**De novo**
									PKD1	*c.11084A>G*	*p.His3695Arg*	*N/A*	*Likely Benign*	*M*
	498/18	father with ADPKD							---	---	---			
	877/18	healthy mother							PKD1	c.11084A>G	p.His3695Arg			
	876/18	older brother with cysts							PKD1	c.12442G>T	p.Glu4148Ter			
	875/18	younger healthy brother							PKD1	c.11084A>G	p.His3695Arg			
124	505/19	Prenatal (TOP)	Normal	No	Normal	Occipital meningocele	Meckel syndrome	Unknown (probably causal finding at	---	---	---	---	---	---

								microarray reported from another laboratory)						
125	1439/18	Prenatal (TOP)	Normal	Multicystic dysplastic kidneys	Normal	Anhydramnios, rectal atresia, intestinal malrotation, Hypoplasia of the urinary bladder	PKD of unknown etiology	Unknown	**PKHD1**	**c.7264T>G**	**p.Cys2422Gly**	**rs201881567**	Likely Pathogenic	**---**
									---	---	---	---	---	---
126	654/19P	Prenatal (TOP)	Normal	Multicystic dysplastic kidneys	Normal	Oligohydramnios	PKD of unknown etiology	Unknown	**TRIM32**	**Suspected deletion of most of the gene**	**N/A**	**N/A**	**N/A**	**P**
									MKS1	**c.491G>A**	**p.Arg164His**	**rs200149256**	Uncertain Significance	**M**
	653/19	healthy father							TRIM32	Suspected deletion of most of the gene	N/A			
	654/19	healthy mother							MKS1	c.491G>A	p.Arg164His			
127	960/16	Perinatal	Not disclosed	Not disclosed	Not disclosed	Not disclosed	ARPKD, EGFR	Unknown	---	---	---	---	---	---
128	645/19	Adolescence	Normal	Kidneys with microcystosis	Normal	No	HNF1B, NPHP	RCAD syndrome	**HNF1B**	**Whole gene deletion**	**N/A**	**N/A**	**---**	**---**

Supplementary Table S2: Group of patients with noncystic nephropathies.

N.	Patient number
dbSNP	ID of variant in the NCBI database of genetic variations
ACMG	Automatic prediction of pathogenicity following criteria by American College of Medical Genetics
Inheritance	P – father, M – mother

aHUS	Atypical hemolytic uremic syndrome
BOR1	Branchiootorenal syndrome
FSGS	Focal segmental glomerulosclerosis
MDCK2/FJHN	Medullary cystic kidney disease 2/familial juvenile hyperuricemic nephropathy

N.	Sample number	Relatives	Clinical diagnosis	Genetic diagnosis	Detected sequence variants					
					Gene	DNA	Protein	dbSNP	ACMG	Inheritance
1b	540/17	---	BOR1	BOR1	**EYA1**	**c.418+5G>A**	**N/A**	**N/A**	Uncertain Significance	**P**
		father with the same diagnosis			EYA1	c.418+5G>A	N/A			
		healthy mother			---	---	---			
		brother with the same diagnosis			EYA1	c.418+5G>A	N/A			
2b	1004/18	---	FSGS	Unknown	*TTC21B*	*c.1697A>G*	*p.His566Arg*	*N/A*	*Likely Benign*	---
3b	1121/18	---	aHUS	aHUS	**C3**	**c.193A>C**	**p.Lys65Gln**	**rs539992721**	Uncertain Significance	
		healthy father			---	---	---			
		healthy mother			C3	c.193A>C	p.Lys65Gln			
		healthy brother			C3	c.193A>C	p.Lys65Gln			
4b	1008/18	---	FSGS	Unknown	---	---	---	---	---	---
5b	1428/18	---	Gitelman syndrome	Gitelman syndrome	**SLC12A3**	**c.1315G>A**	**p.Gly439Ser**	**rs759377924**	Likely Pathogenic	
					SLC12A3	**c.1946C>T**	**p.Thr649Met**	**rs145337602**	Pathogenic	
6b	1427/18	---	Gitelman syndrome	Gitelman syndrome	**SLC12A3**	**c.1315G>A**	**p.Gly439Ser**	**rs759377924**	Likely Pathogenic	
					SLC12A3	**c.1946C>T**	**p.Thr649Met**	**rs145337602**	Pathogenic	
7b	963/18	---	FSGS	Unknown	---	---	---	---	---	---
8b	1372/18	---	Chronic renal insufficiency	FSGS	**INF2**	**c.653G>A**	**p.Arg218Gln**	**rs267607183**	Likely Pathogenic	**---**
9b	1127/18	---	aHUS	Unknown	---	---	---	---	---	---
10b	1126/18	---	aHUS	Unknown	*SLC3A1*	*c.1400T>C*	*p.Met467Thr*	*rs121912691*	*Likely Pathogenic*	---
11b	1366/18	---	Chronic renal insufficiency	Unknown	---	---	---	---	---	---
12b	1433/18	---	FSGS	FSGS	**ACTN4**	**c.475T>C**	**p.Ser159Pro**	**N/A**	Uncertain Significance	**---**
13b	1484/18	---	FSGS	Unknown	---	---	---	---	---	---
14b	1333/18	---	FSGS	Unknown	---	---	---	---	---	---
15b	1448/18	---	Gitelman syndrome	Unknown	**CLCNKB**	**Whole gene deletion**	**N/A**	**N/A**	N/A	**---**
					---	---	---	---	---	---
16b	1669/18	---	C3 glomerulopathy	Unknown	---	---	---	---	---	---
17b	1103/17	---	Nail-patella syndrome	Nail-patella syndrome	**LMX1B**	**c.543delC**	**p.Asp182ThrfsTer6**	**rs1114167362**	Pathogenic	**---**
18b	1629/18	---	aHUS	Unknown	**PAX2**	**c.320C>T**	**p.Pro107Leu**	**rs774231216**	Likely Pathogenic	**---**
19b	1628/18	---	aHUS	Unknown	---	---	---	---	---	---
20b	1635/18	---	aHUS	aHUS	**CFH**	**c.1352C>G**	**p.Ser451Ter**	**N/A**	Likely Pathogenic	**---**
21b	143/18	---	Chronic renal insufficiency	MDCK2/FJHN	**UMOD**	**c.782G>C**	**p.Arg261Pro**	**N/A**	Uncertain Significance	**---**

Supplementary Table S3: Full list of genes contained in panel version number 1

ACTN4	NM_004924
AHI1	NM_017651
ALMS1	NM_015120
ANKS6	NM_173551
ANLN	NM_018685
APOL1	NM_003661
ARHGDIA	NM_001185077
ARL13B	NM_182896
ARL6	NM_032146
B9D1	NM_015681
B9D2	NM_030578
BBIP1	NM_001195304
BBS1	NM_024649
BBS10	NM_024685
BBS12	NM_152618
BBS2	NM_031885
BBS4	NM_033028
BBS5	NM_152384
BBS7	NM_176824
BBS9	NM_198428
BICC1	NM_001080512
BVES	NM_007073
C3	NM_000064
CC2D2A	NM_001080522
CD2AP	NM_012120
CD46	NM_002389
CEP164	NM_014956
CEP290	NM_025114
CEP41	NM_018718
CFB	NM_001710
CFI	NM_000204
CLCNKB	NM_000085
COL4A3	NM_000091
COL4A4	NM_000092
COL4A5	NM_000495
COQ8B	NM_024876
CPLANE1	NM_023073
CRB2	NM_173689
DGKE	NM_003647
DYNC2H1	NM_001080463
EMP2	NM_001424
EYA1	NM_000503
GDNF	NM_000514
GFRA1	NM_005264
GLIS2	NM_032575
HNF1B	NM_000458
IFT140	NM_014714
IFT172	NM_015662
IFT27	NM_001177701
IFT80	NM_020800
INF2	NM_022489
INVS	NM_014425
IQCB1	NM_001023570
KCNJ1	NM_000220
KIF14	NM_014875
KIF7	NM_198525
LAMC1	NM_002292
LMX1B	NM_002316
LZTFL1	NM_020347
MKKS	NM_018848
MKS1	NM_017777
MUC1	NM_002456
MYH9	NM_002473
MYO1E	NM_004998
NAT8	NM_000169
NEK8	NM_178170
NPHP1	NM_000272
NPHP3	NM_153240
NPHP4	NM_015102
NPHS1	NM_004646
NPHS2	NM_014625
OFD1	NM_003611
PAX2	NM_003990
PEX1	NM_000466
PEX10	NM_153818
PEX11B	NM_003846
PEX13	NM_002618
PEX14	NM_004565
PEX16	NM_057174
PEX19	NM_002857
PEX2	NM_000318
PEX26	NM_017929
PEX3	NM_003630
PEX5	NM_001300789
PEX6	NM_000287
PKD1	NM_001009944
PKD2	NM_000297
PKHD1	NM_138694
PLCE1	NM_016341
PMPCA	NM_019892
PTPRO	NM_030667
REN	NM_000537
RET	NM_020975
RPGRIP1L	NM_015272
SDCCAG8	NM_006642
SLC12A1	NM_000338

SLC12A3	NM_000339
TCTN1	NM_001082538
TCTN2	NM_024809
TCTN3	NM_015631
THBD	NM_000361
TMEM138	NM_001044385
TMEM216	NM_016499
TMEM231	NM_001077416
TMEM237	NM_152388
TMEM67	NM_153704
TRIM32	NM_012210
TRPC6	NM_004621
TSC1	NM_000368
TSC2	NM_000548
TTC21B	NM_024753
TTC8	NM_144596
UMOD	NM_003361
VHL	NM_000551
WDPCP	NM_015910
WDR19	NM_025132
WT1	NM_024426
ZNF423	NM_015069

Supplementary Table S4: Full list of genes contained in panel version number 2

ACE	NM_000789.3
ACTN4	NM_001322033.1
AGT	NM_000029.3
AGTR1	NM_000685.4
AGXT	NM_000030.2
AHI1	NM_017651.4
ANKS6	NM_173551.4
ANLN	NM_018685.4
APOL1	NM_145343.2
AQP2	NM_000486.5
ARHGDIA	NM_004309.5
ARL13B	NM_182896.2
ARL6	NM_032146.5
ATP6V0A4	NM_020632.2
ATP6V1B1	NM_001692.3
AVP	NM_000490.4
AVPR2	NM_000054.4
B9D1	NM_015681.4
B9D2	NM_030578.3
BBIP1	NM_001195305.1
BBS1	NM_024649.4
BBS10	NM_024685.3
BBS12	NM_152618.2
BBS2	NM_031885.3
BBS4	NM_033028.4
BBS5	NM_152384.2
BBS7	NM_018190.3
BBS9	NM_001033604.1
BICC1	NM_001080512.2
BMP4	NM_001202.5
BSND	NM_057176.2
C3	NM_000064.3
CASR	NM_000388.3
CC2D2A	NM_001080522.2
CD2AP	NM_012120.2
CD46	NM_172361.2
CEP104	NM_014704.3
CEP164	NM_014956.4
CEP41	NM_018718.2
CFB	NM_001710.5
CFH	NM_000186.3
CFHR1	NM_002113.2
CFHR3	NM_021023.5
CFHR5	NM_030787.3
CFI	NM_000204.4
CLCNKA	NM_001042704.1
CLCNKB	NM_000085.4
CLDN16	NM_006580.3
CLDN19	NM_148960.2
COL4A1	NM_001303110.1
COL4A2	NM_001846.3
COL4A3	NM_000091.4
COL4A4	NM_000092.4
COL4A5	NM_000495.4
COL4A6	NM_001287758.1
COQ2	NM_015697.8
COQ6	NM_182476.2
COQ8B	NM_024876.3
CPLANE1	NM_023073.3
CRB2	NM_173689.6
DGKE	NM_003647.2
DSTYK	NM_015375.2
DYNC2H1	NM_001080463.1
DZIP1L	NM_173543.2
EMP2	NM_001424.5
EYA1	NM_000503.5
FGF20	NM_019851.2
FN1	NM_212476.2
FRAS1	NM_001166133.1
FREM1	NM_144966.5
GANAB	NM_198334.2
GATA3	NM_001002295.1
GLIS2	NM_032575.2
GRHPR	NM_012203.1
GRIP1	NM_001178074.1
HNF1B	NM_000458.3
HOGA1	NM_138413.3
CHD1L	NM_004284.5
IFT80	NM_020800.2
INF2	NM_022489.3
INVS	NM_014425.4
IQCB1	NM_001023570.3
ITGA8	NM_003638.2
KCNJ1	NM_000220.4
KIF7	NM_198525.2
LAMC1	NM_002292.3
LMX1B	NM_001174146.1
LRP5	NM_002335.3
LZTFL1	NM_020347.3
MAGED2	NM_014599.5
MKKS	NM_018848.3
MKS1	NM_017777.3
MUC1	NM_002456.5
MYH9	NM_002473.5
MYO1E	NM_004998.3
NEK8	NM_178170.2

NPHP1	NM_000272.3
NPHP3	NM_153240.4
NPHP4	NM_001291593.1
NPHS1	NM_004646.3
NPHS2	NM_001297575.1
NUP107	NM_020401.3
NUP205	NM_015135.2
NUP93	NM_014669.4
OFD1	NM_003611.2
PAX2	NM_000278.4
PDSS2	NM_020381.3
PKHD1	NM_138694.3
PLCE1	NM_016341.3
PMPCA	NM_019892.5
PRKCSH	NM_001001329.2
PTPRO	NM_002848.3
REN	NM_000537.3
RET	NM_020975.5
ROBO2	NM_002942.4
RPGRIP1L	NM_015272.4
SALL1	NM_002968.2
SDCCAG8	NM_006642.4
SEC63	NM_007214.4
SIX1	NM_005982.3
SIX2	NM_016932.4
SIX5	NM_175875.4
SLC12A1	NM_000338.2
SLC12A3	NM_000339.2
SLC34A1	NM_003052.4
SLC3A1	NM_000341.3
SLC4A1	NM_000342.3
SLC7A9	NM_001126335.1
SLC9A3R1	NM_004252.4
SOX17	NM_022454.3
TCTN1	NM_024549.5
TCTN2	NM_024809.4
TCTN3	NM_015631.5
THBD	NM_000361.2
TMEM138	NM_016464.4
TMEM216	NM_016499.5
TMEM231	NM_001077416.2
TMEM237	NM_152388.3
TMEM67	NM_153704.5
TRAF3IP1	NM_015650.3
TRIM32	NM_012210.3
TRPC6	NM_004621.5
TSC1	NM_000368.4
TSC2	NM_000548.4
TTC21B	NM_024753.4
TTC8	NM_144596.3
UMOD	NM_001008389.2
UPK3A	NM_006953.3
VHL	NM_000551.3
WDPCP	NM_015910.6
WDR19	NM_001317924.1
WT1	NM_000378.5
ZNF423	NM_015069.4

Supplementary Table S5: Quality metrics of panel sequencing

PCT_TARGET_BASES_10X	The fraction of all target bases achieving 10X or greater coverage.
PCT_TARGET_BASES_30X	The fraction of all target bases achieving 30X or greater coverage.
PCT_TARGET_BASES_50X	The fraction of all target bases achieving 50X or greater coverage.
PCT_TARGET_BASES_100X	The fraction of all target bases achieving 100X or greater coverage.
MEDIAN_TARGET_COVERAGE	The median coverage of a target region.
MEAN_TARGET_COVERAGE	The mean coverage of a target region.
MAX_TARGET_COVERAGE	The maximum coverage of reads that mapped to target regions of an experiment.
TOTAL_READS	The total number of reads in the SAM or BAM file examined.
ON_TARGET_BASES	The number of PF_BASES_ALIGNED that are mapped to a targeted region of the genome.
PCT_OFF_BAIT	The fraction of PF_BASES_ALIGNED that are mapped away from any baited region, OFF_BAIT_BASES/PF_BASES_ALIGNED.

Panel v1										
Sample	PCT_TARGET_BASES_10X	PCT_TARGET_BASES_30X	PCT_TARGET_BASES_50X	PCT_TARGET_BASES_100X	MEDIAN_TARGET_COVERAGE	MEAN_TARGET_COVERAGE	MAX_TARGET_COVERAGE	TOTAL_READS	ON_TARGET_BASES	PCT_OFF_BAIT
889-12	0.988872	0.975577	0.957096	0.885695	187.0	182.534007	545.0	2371582.0	125979678.0	0.321868
716-15	0.986802	0.967917	0.938691	0.733043	122.0	120.060344	311.0	1413352.0	82862168.0	0.3029
640-15	0.986611	0.969651	0.943826	0.74838	124.0	123.266974	321.0	1441036.0	85075291.0	0.300941
885-12	0.986043	0.9701	0.944627	0.820353	149.0	144.675831	408.0	1791240.0	99851063.0	0.310902
884-12	0.985922	0.971933	0.949991	0.855488	159.0	156.364834	456.0	1905015.0	107918474.0	0.312243
1479-12	0.988088	0.979353	0.968848	0.918775	200.0	207.255224	622.0	2535499.0	143041545.0	0.320356
446-13	0.987768	0.975015	0.947668	0.747774	130.0	133.371499	489.0	1646876.0	92049141.0	0.330717
1340-13	0.988503	0.977716	0.964273	0.905573	200.0	201.044251	544.0	2393991.0	138754912.0	0.304958
943-12	0.986981	0.975561	0.958907	0.88728	163.0	159.118378	417.0	1896584.0	109818890.0	0.308379
886-12	0.986937	0.973055	0.954897	0.895106	200.0	208.961153	628.0	2598645.0	144218928.0	0.31217
160-15	0.9887	0.976084	0.959756	0.895843	173.0	171.51426	499.0	2119180.0	118374168.0	0.323891
765-12	0.988393	0.975197	0.955753	0.873233	161.0	157.408126	432.0	1991811.0	108638524.0	0.312783
001-16	0.990194	0.980924	0.970147	0.9257	200.0	195.067112	453.0	2339836.0	134629664.0	0.254389
1040-16	0.990963	0.983949	0.974498	0.931516	200.0	203.8827	581.0	2375817.0	140713927.0	0.249794
1178-12	0.992705	0.985343	0.978184	0.950359	200.0	225.060637	526.0	2520432.0	155330325.0	0.235049
194-16	0.988884	0.980137	0.968737	0.917276	193.0	187.33959	481.0	2225362.0	129296352.0	0.243251
282-16	0.990617	0.983362	0.974373	0.938486	200.0	213.738762	518.0	2478184.0	147516295.0	0.256579
383-16	0.988765	0.972818	0.959071	0.908398	184.0	180.053866	573.0	2476296.0	124267957.0	0.256826
407-16	0.989164	0.978991	0.964649	0.881376	164.0	156.620762	333.0	1669182.0	108095108.0	0.221192
472-16	0.991018	0.983394	0.973049	0.94137	200.0	205.968008	545.0	2415317.0	142153146.0	0.24517
515-15	0.986567	0.96999	0.948419	0.838385	149.0	142.234584	328.0	1588230.0	98166185.0	0.245042
69-14	0.992408	0.985757	0.978914	0.952113	200.0	244.665696	570.0	2806581.0	168861168.0	0.239812

797-13	0.989759	0.980467	0.970993	0.923591	200.0	192.503919	418.0	2182976.0	132860622.0	0.235091
883-12	0.990681	0.982606	0.972233	0.931975	191.0	184.816598	408.0	2122919.0	127555056.0	0.241564
887-12	0.990414	0.981305	0.970548	0.905066	177.0	169.474523	376.0	1872557.0	116966401.0	0.234751
889-13	0.986057	0.969111	0.943876	0.790443	132.0	127.179879	329.0	1446780.0	87775864.0	0.236131
942-16	0.991802	0.984216	0.976817	0.940521	200.0	209.783013	478.0	2387289.0	144786152.0	0.249524
1236-12	0.991808	0.983865	0.974637	0.922048	186.0	179.571965	428.0	2288280.0	123935363.0	0.300736
1458-16	0.990178	0.979178	0.96773	0.906474	170.0	163.699638	375.0	2129649.0	112980743.0	0.300688
236-16	0.99107	0.978103	0.959732	0.828682	129.0	125.340152	269.0	1519035.0	86506138.0	0.277406
352-17	0.990689	0.980954	0.969799	0.910093	165.0	158.922792	364.0	1932752.0	109683902.0	0.272343
419-17	0.993894	0.984459	0.972556	0.914597	170.0	164.651394	377.0	1970145.0	113637617.0	0.271702
449-16	0.992172	0.981534	0.966772	0.865032	152.0	146.983224	337.0	1765541.0	101443559.0	0.264779
540-17	0.99055	0.981053	0.971671	0.915453	184.0	175.579991	379.0	2136099.0	121180218.0	0.287641
541-17	0.985748	0.967172	0.930003	0.608849	107.0	103.214566	255.0	1268369.0	71235700.0	0.277264
606-17	0.991299	0.981693	0.969173	0.897256	174.0	166.31303	391.0	2064813.0	114784430.0	0.291644
759-17	0.992992	0.985275	0.976097	0.939524	197.0	191.477974	467.0	2369938.0	132152545.0	0.279374
770-17	0.992682	0.98375	0.975277	0.916569	171.0	164.916648	352.0	1985186.0	113820688.0	0.277617
853-17	0.989314	0.976942	0.959806	0.861331	142.0	136.582689	316.0	1686286.0	94265411.0	0.279476
859-16	0.994032	0.987302	0.979456	0.943431	200.0	198.767831	450.0	2405264.0	137183793.0	0.280665
88-17	0.988977	0.975316	0.955552	0.839573	136.0	132.048823	366.0	1756998.0	91136268.0	0.275713
952-16	0.990643	0.976528	0.956053	0.799735	130.0	124.835141	301.0	1545242.0	86157594.0	0.293395
Panel v2										
Sample	PCT_TARGET_BASES_10X	PCT_TARGET_BASES_30X	PCT_TARGET_BASES_50X	PCT_TARGET_BASES_100X	MEDIAN_TARGET_COVERAGE	MEAN_TARGET_COVERAGE	MAX_TARGET_COVERAGE	TOTAL_READS	ON_TARGET_BASES	PCT_OFF_BAIT
1004-18	0.997713	0.993594	0.987521	0.938218	151.0	148.174779	264.0	3773807.0	78341784.0	0.484862

1008-18	0.997059	0.991356	0.982928	0.838248	123.0	121.279379	221.0	2938424.0	64121863.0	0.452937
1023-18	0.997571	0.990971	0.98101	0.829834	123.0	120.906151	229.0	2865421.0	63924533.0	0.46021
1121-18	0.994791	0.969641	0.887286	0.100694	77.0	75.21318	171.0	1801460.0	39766111.0	0.466868
1126-18	0.997218	0.99137	0.981107	0.874892	142.0	137.07282	249.0	3531570.0	72472045.0	0.492774
1127-18	0.99767	0.993153	0.987511	0.951253	163.0	160.235219	307.0	4040132.0	84718283.0	0.496682
1252-18	0.996715	0.989821	0.981793	0.864329	128.0	125.683892	232.0	3080876.0	66450582.0	0.451078
1333-18	0.996007	0.989822	0.980691	0.898657	144.0	140.502391	323.0	3731934.0	74285300.0	0.501679
1366-18	0.998198	0.994116	0.987611	0.928602	173.0	167.28559	291.0	4257840.0	88445899.0	0.499374
1372-18	0.997583	0.992675	0.986004	0.927119	150.0	147.075572	257.0	3523620.0	77760620.0	0.457962
1427-18	0.997176	0.99057	0.971832	0.766896	118.0	114.384873	206.0	2869157.0	60476655.0	0.466692
1428-18	0.997397	0.992671	0.985482	0.915211	164.0	158.39886	331.0	4062962.0	83747378.0	0.488006
1433-18	0.996836	0.992631	0.984419	0.900485	150.0	145.674017	272.0	3721785.0	77019601.0	0.491086
1448-18	0.997891	0.993845	0.988845	0.95747	180.0	175.6963	335.0	4363328.0	92892742.0	0.483376
1484-18	0.994901	0.988463	0.981296	0.895836	136.0	132.986121	228.0	3447705.0	70311358.0	0.499437
963-18	0.998082	0.994403	0.987844	0.923771	164.0	159.412661	291.0	3955291.0	84283387.0	0.474455
1103-17	0.997029	0.989003	0.977133	0.829777	124.0	122.402172	252.0	2609707.0	64715497.0	0.348892
1238-18	0.992151	0.985378	0.971852	0.830827	128.0	124.654797	246.0	2607217.0	65906487.0	0.330644
1287-18	0.997806	0.992451	0.986091	0.943546	162.0	161.217311	341.0	3520211.0	85237527.0	0.384266
1350-18	0.997884	0.991971	0.980685	0.900006	181.0	180.016077	441.0	3745032.0	95176660.0	0.368086
1351-18	0.995854	0.985605	0.973706	0.860357	149.0	148.90851	372.0	3084862.0	78729716.0	0.367365
143-18	0.996024	0.987706	0.976377	0.862825	132.0	129.482862	250.0	2869194.0	68459143.0	0.357144
1563-18	0.993465	0.962019	0.858227	0.088025	74.0	72.496317	167.0	1527168.0	38329673.0	0.348387
1628-18	0.997059	0.991812	0.98491	0.91812	166.0	160.784157	300.0	3445341.0	85008513.0	0.355402
1629-18	0.996541	0.988773	0.978067	0.892045	159.0	152.74993	282.0	3380446.0	80760721.0	0.367012
1635-18	0.996628	0.990901	0.981048	0.900401	154.0	149.888811	335.0	3201826.0	79248013.0	0.357385
1668-18	0.995761	0.989622	0.979855	0.883982	132.0	130.322397	249.0	2914182.0	68903015.0	0.377954
1669-18	0.993664	0.988287	0.979959	0.914965	167.0	161.334636	324.0	3600310.0	85299558.0	0.390222

1728-18	0.996486	0.987324	0.974029	0.766909	117.0	115.024528	227.0	2437580.0	60814848.0	0.339015
1737-18	0.997611	0.993471	0.98763	0.937422	180.0	175.432504	348.0	3703952.0	92753270.0	0.351008
863-18	0.998827	0.995822	0.991708	0.959468	200.0	203.922722	490.0	4209639.0	107816390.0	0.336744
1439-18	0.996416	0.986473	0.966244	0.470252	98.0	97.116789	219.0	2026072.0	51346812.0	0.367691
505-19	0.998493	0.995669	0.991901	0.947457	182.0	178.592532	340.0	4082837.0	94424015.0	0.417375
645-19	0.997333	0.993654	0.989287	0.964811	182.0	177.987339	313.0	4094369.0	94104042.0	0.419738
653-19	0.998644	0.996098	0.993713	0.972573	200.0	225.716573	480.0	4901943.0	119339061.0	0.41825
654-19-M	0.997528	0.993323	0.988534	0.962028	175.0	173.531371	329.0	3850887.0	91748118.0	0.402447
654-19-P	0.997326	0.991597	0.985395	0.923066	181.0	174.300924	331.0	4087829.0	92154990.0	0.423955

Supplementary Table S6: Summary of molecular genetic analyses executed in individual patients

+ Yes

- No

		PKHD1 **sequencing**	**MLPA** ***PKHD1***	**MLPA** ***HNF1B***	**Panel sequencing**	***PKD1*** **sequencing**
Group 1: cystic kidney diseases						
1	885/12	+	+	+	+	+
2	884/12	+	+	+	+	+
3	1567/12	+	-	-	-	-
4	600/13	+	+	-	-	-
5	893/13	+	-	-	-	-
6	1358/12	+	-	-	-	-
7	1177/12	+	-	-	-	-
8	1388/12	+	-	-	-	-
9	1479/12	+	+	+	+	+
10	446/13	+	+	+	+	+
11	1513/13	+	-	-	-	-
12	1359/12	+	-	-	-	-
13	889/13	+	+	+	+	+
14F	832/13	+	+	+	-	-
14M	891/13	+	+	+	-	-
15	91192/13	+	+	+	-	-
16	124/14	+	-	-	-	-
17	461/14	+	+	-	-	-
18	460/14	+	+	-	-	-
19	1340/13	+	+	+	+	+
20	1371/13	+	+	-	-	+
21	605/14	+	+	+	-	-
22	883/14	+	+	-	-	-
23	1085/14	+	-	-	-	-
24	943/12	+	+	+	+	+
25	797/13	+	+	+	+	+
26	701/14	+	-	+	-	-
27	1052/14	+	-	-	-	-
28	1006/14	+	-	-	-	-
29	1178/12	+	+	+	+	+
30	886/12	+	+	+	+	+
31	1360/12	+	+	+	-	-
32	887/12	+	+	+	+	+
33F	078/15	+	-	-	-	-
33M	079/15	+	-	-	-	-
34	160/15	+	+	+	+	+
35	888/12	+	-	-	-	-
36	865/12	+	-	-	-	-
37	863/12	+	-	-	-	-

38	883/12	+	+	-	+	+
39	765/12	+	+	+	+	+
40	889/12	+	+	+	+	+
41	864/12	+	-	-	-	-
42	637/15	+	-	-	-	-
43	640/15	+	+	+	+	+
44	378/15	+	-	-	-	-
45F	471/15	+	+	+	-	-
45M	467/15	+	+	+	-	-
46	642/15	+	+	-	-	-
47	1179/14	+	+	-	-	-
48	589/15	+	+	-	-	-
49	1011/15	+	-	-	-	-
50	1294/15	+	-	-	-	-
51F	1291/15	+	-	-	-	-
51M	1290/15	+	-	-	-	-
52	094/16	+	+	+	-	-
53	021/16	+	-	-	-	-
54	1439/15	+	+	+	-	-
55	194/16	-	-	-	+	+
56	1040/16	-	-	-	+	+
57	001/16	-	-	-	+	+
58	282/16	-	-	-	+	+
59	69/14	-	-	-	+	+
60	383/16	-	-	-	+	+
61	472/16	-	-	-	+	+
62	407/16	-	-	-	+	+
63	515/15	-	-	-	+	+
64	419/17	-	-	-	+	+
65	859/16	-	-	-	+	+
66	952/16	-	-	-	+	+
67	449/16	-	-	-	+	+
68	236/16	-	-	-	+	+
69	352/17	-	-	-	+	+
70	770/17	-	-	-	+	+
71	759/17	-	-	-	+	+
72	606/17	-	-	-	+	+
73	540/17	-	-	-	+	+
74	541/17	-	-	-	+	+
75	1458/16	-	-	-	+	+
76	88/17	-	-	-	+	-
77	853/17	-	-	-	+	+
78	1023/18	-	-	-	+	-

79	1252/18	-	-	-	+	+
80	1238/18	-	-	-	+	-
81	1563/18	-	-	-	+	-
82	1728/18	-	-	-	+	-
83	1350/18	-	-	-	+	-
84	1351/18	-	-	-	+	-
85	1737/18	-	-	-	+	-
86	1668/18	-	-	-	+	+
87	1287/18	-	-	-	+	-
88	863/18	-	-	-	+	-
89	1048/17	-	-	-	-	+
90	1000/17	-	-	-	-	+
91	191/17	-	-	-	-	+
92	535/17	-	-	-	-	+
93	1500/17	-	-	-	-	+
94	1505/17	-	-	-	-	+
95	16/18	-	-	-	-	+
96	1244/17	-	-	-	-	+
97	1295/17	-	-	-	-	+
98	1344/17	-	-	-	-	+
99	1339/17	-	-	-	-	+
100	1124/17	-	-	-	-	+
101	562/16	-	-	-	-	+
102	497/18	-	-	-	-	+
103	619/18	-	-	-	-	+
104	1184/18	-	-	-	-	+
105	---	-	-	-	-	+
106	1627/17	-	-	-	-	+
107	1490/18	-	-	-	-	+
108	001/19	-	-	-	-	+
109	1724/18	-	-	-	-	+
110	442/15	-	-	-	-	+
111	775/15	-	-	-	-	+
112	1345/18	-	-	-	-	+
113	696/19	-	-	-	-	+
114	388/18	-	-	-	-	+
115	312/19	-	-	-	-	+
116	715/19	-	-	-	-	+
117	862/19	-	-	-	-	+
118	825/18	-	-	-	-	+
119	882/19	-	-	-	-	+
120	1612/18	-	-	-	-	+
121	31/19	-	-	-	-	+

122	1155/17	+	-	-	-	-
123	916/15	+	+	+	+	+
124	505/19	-	-	-	+	-
125	1439/18	-	-	-	+	-
126	654/19P	-	-	-	+	-
127	960/16	-	-	+	+	+
128	645/19	-	-	-	+	-
Group 2: noncystic kidney diseases						
1	540/17	-	-	-	+	+
2	1004/18	-	-	-	+	-
3	1121/18	-	-	-	+	-
4	1008/18	-	-	-	+	-
5	1428/18	-	-	-	+	-
6	1427/18	-	-	-	+	-
7	963/18	-	-	-	+	-
8	1372/18	-	-	-	+	-
9	1127/18	-	-	-	+	-
10	1126/18	-	-	-	+	-
11	1366/18	-	-	-	+	-
12	1433/18	-	-	-	+	-
13	1484/18	-	-	-	+	-
14	1333/18	-	-	-	+	-
15	1448/18	-	-	-	+	-
16	1669/18	-	-	-	+	-
17	1103/17	-	-	-	+	-
18	1629/18	-	-	-	+	-
19	1628/18	-	-	-	+	-
20	1635/18	-	-	-	+	-
21	143/18	-	-	-	+	-